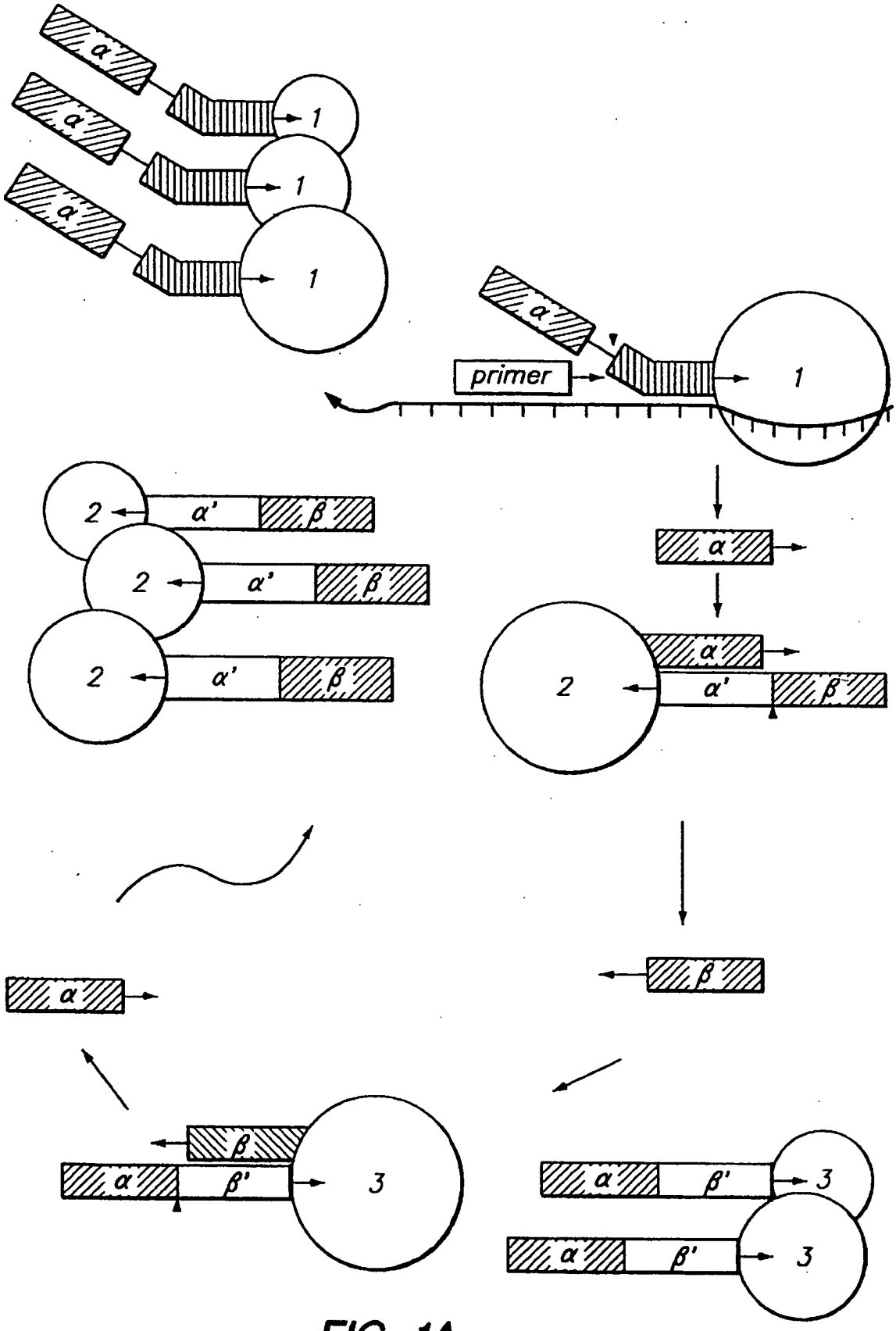



FIG. 1A

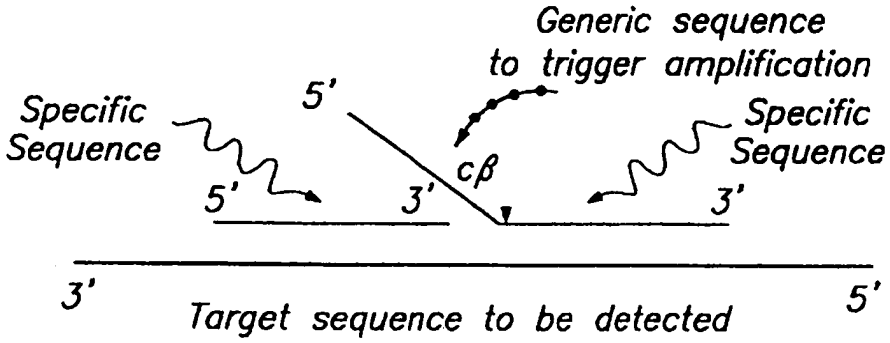



**FIG. 1B** PART ONE: TRIGGER REACTION

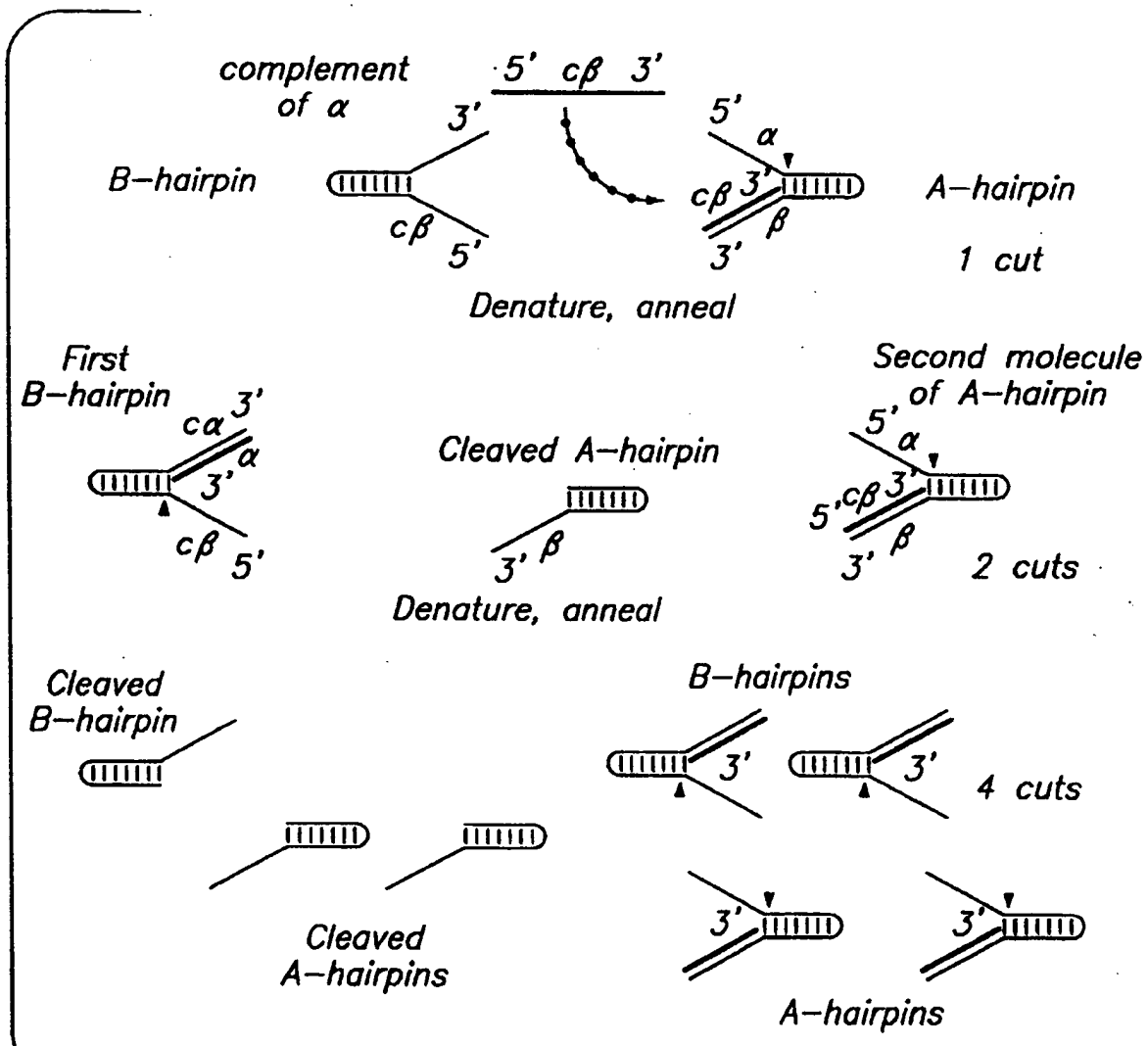
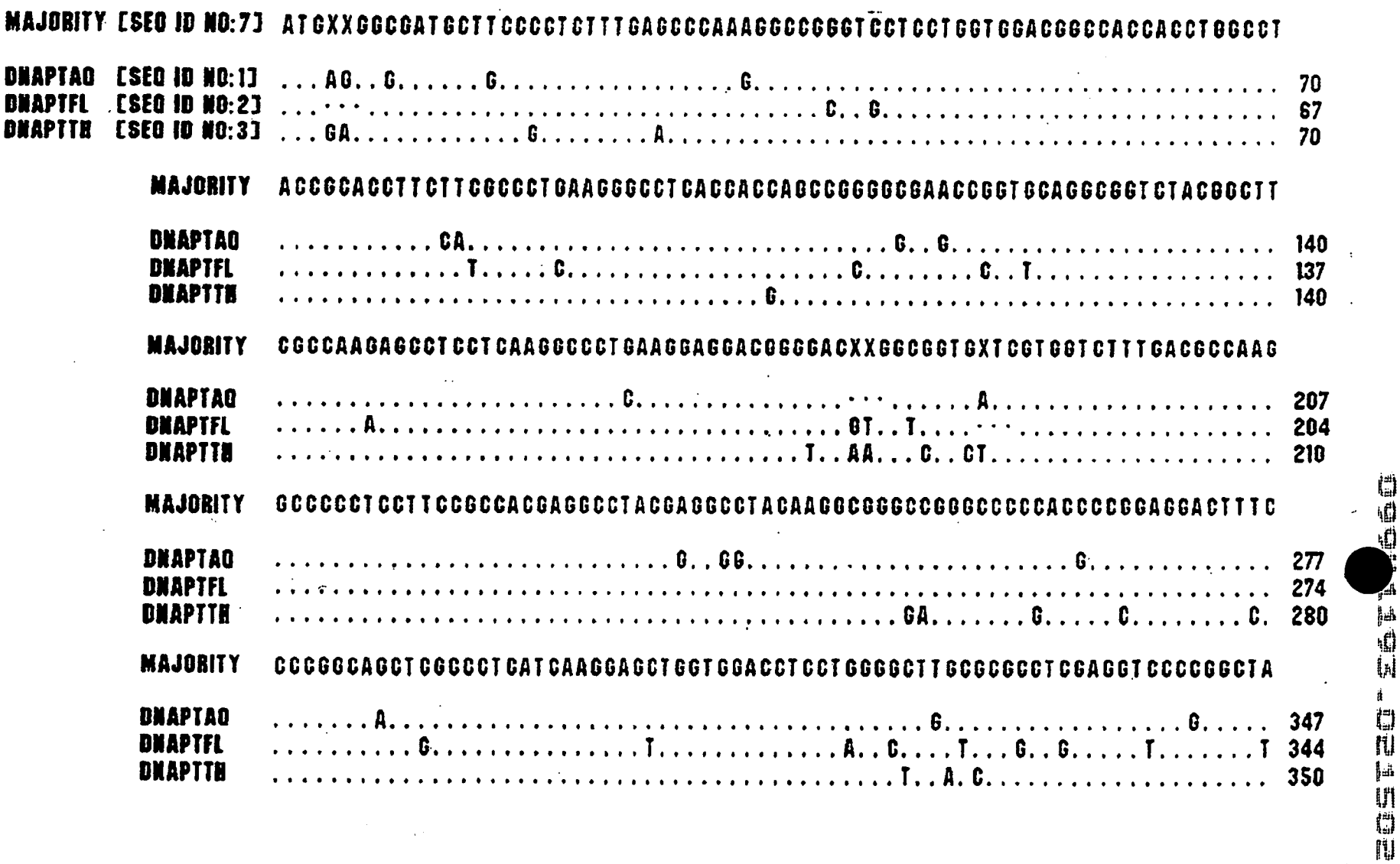



**FIG. 1C** PART TWO: DETECTION REACTION

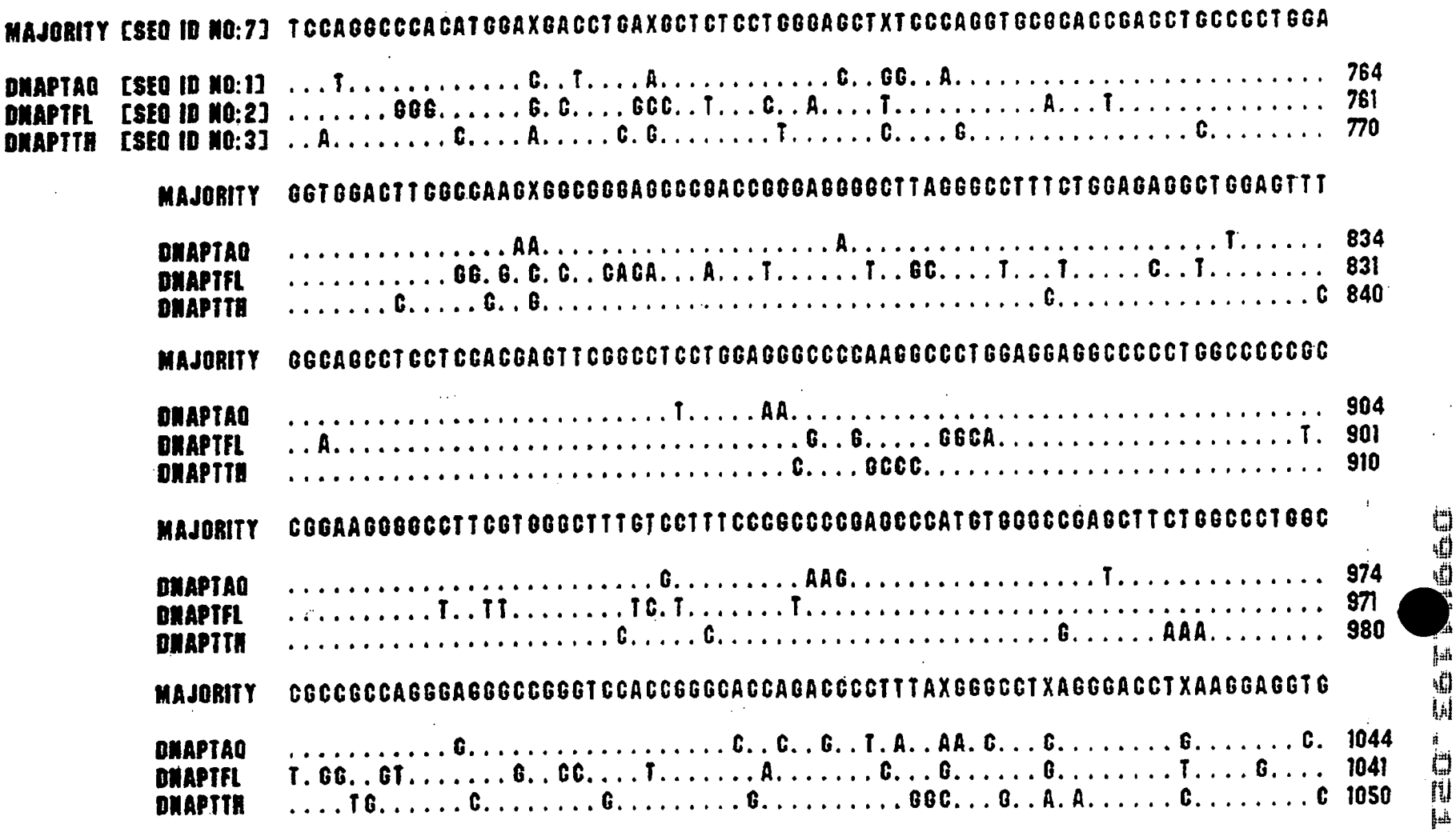
# FIG. 2A


MAJORITY [SEQ ID NO:7] ATGXXGGCGATGCTTCCCCTCTTTGAGCCCAAAGGCCGGGTCCTCCTGGTGGACGGCCACCACCTGGCCT
DNAPTAQ [SEQ ID NO:1] ...AG..G.....G.....G..... 70
DNAPTFL [SEQ ID NO:2] .....C..G..... 67
DNAPTTH [SEQ ID NO:3] ...GA.....G.....A..... 70
MAJORITY ACCGCACCTTCTTCGCCCTGAAGGGCCTCACCACCAGCCGGGGCGAACCGGTGCAGGCGGTCTACGGCTT
DNAPTAQ .....CA.....G..G..... 140
DNAPTFL .....T.....C.....C.....C..T..... 137
DNAPTTH .....G..... 140
MAJORITY CGCCAAGAGCCTCCTCAAGGCCCTGAAGGAGGACGGGGACXXGGCGGTGXTCGTGGTCTTTGACGCCAAG
DNAPTAQ .....C.....A..... 207
DNAPTFL .....A.....GT..T..... 204
DNAPTTH .....T..AA...C..CT..... 210
MAJORITY GCCCCCTCCTTCCGCCACGAGGCCTACGAGGCCTACAAGGCGGGCCGGGCCCCCACCCCGGAGGACTTTC
DNAPTAQ .....G..GG.....G..... 277
DNAPTFL ..... 274
DNAPTTH .....GA.....G.....C.....C. 280
MAJORITY CCCGGCAGGCTCGCCCTCATCAAGGAGCTGGTGGACCTCCTGGGGCTTGCGCGCCTCGAGGTCCCCGGCTA
DNAPTAQ .....A.....G.....G..... 347
DNAPTFL .....G.....T.....A..C....T...G..G.....T.....T 344
DNAPTTH .....T..A.C..... 350

# FIG. 2B

```
MAJORITY [SEQ ID NO:7] CGAGGCGGACGACGTXGTGGCCACCCTGGCCAAGAAGGCGGAAAAGGAGGGGTACGAGGTGCGCATCCTC

DNAPTAQ  [SEQ ID NO:1] ..............C.......G.............................C........C.........  417
DNAPTFL  [SEQ ID NO:2] T.............G.................CG.....................................  414
DNAPTTH  [SEQ ID NO:3] ..............T..C.....................................................  420

MAJORITY ACCGCCGACCGCGACCTCTACCAGCTCCTTTCCGACCGCATCGCCGTCCTCCACCCCGAGGGCTACCTCA

DNAPTAQ  .........AAA.....T.........................CA.............................  487
DNAPTFL  ..T.............................G..G.........A..........T...........G.  484
DNAPTTH  ........................A...G.C.........G.....................CC......  490

MAJORITY TCACCCCGGCGTGGCTTTGGGAGAAGTACGGCCTGAGGCCGGAGCAGTGGGTGGACTACCGGGCCCTGGC

DNAPTAQ  ..........C...........A.................C..C.......CC..............A.  557
DNAPTFL  ..................AC..............C.C.................................  554
DNAPTTH  .........A.........................C.....................T...C.....C.T  560

MAJORITY GGGGGACCCCTCCGACAACCTCCCCGGGGTCAAGGGCATCGGGGAGAAGACCGCCCXGAAGCTCCTCXAG

DNAPTAQ  C......GAG...........T.............................G..GAG......T..GG..  627
DNAPTFL  ............G..T...A.........G.....................A..G....A..CGC  624
DNAPTTH  ..................................................TC.........A..  630

MAJORITY GAGTGGGGGAGCCTGGAAAACCTCCTCAAGAACCTGGACCGGGTGAAGCCCGC···CXTCCGGGAGAAGA

DNAPTAQ  ..................GC.....................C.............A.............  694
DNAPTFL  ........................T..C..C.........A..........T.···.T.G.........C  691
DNAPTTH  ........A.....................................A.....A.AAAA.G.............  700
```

# FIG. 2C


MAJORITY [SEQ ID NO:7] TCCAGGCCCACATGGAXGACCTGAXGCTCTCCTGGGAGCTXTCCCAGGTGCGCACCGACCTGCCCCTGGA
DNAPTAQ [SEQ ID NO:1] ...T.....C..T....A.....C..GG..A..... 764
DNAPTFL [SEQ ID NO:2] .....GGG.....G.C....GCC..T...C..A....T.....A...T..... 761
DNAPTTH [SEQ ID NO:3] ..A.....C....A.....C.G.....T.....C....G.....C..... 770
MAJORITY GGTGGACTTCGCCAAGXGGCGGGAGCCCGACCGGGAGGGGCTTAGGGCCTTTCTGGAGAGGCTGGAGTTT
DNAPTAQ .....AA.....A.....T..... 834
DNAPTFL .....GG.G.C.C..CACA...A...T.....T..GC....T...T.....C..T..... 831
DNAPTTH .....C.....C..G.....C.....C 840
MAJORITY GGCAGCCTCCTCCACGAGTTCGGCCTCCTGGAGGGCCCCAAGGCCCTGGAGGAGGCCCCCTGGCCCCCGC
DNAPTAQ .....T.....AA..... 904
DNAPTFL ..A.....G..G.....GGCA.....T. 901
DNAPTTH .....C....GCCC..... 910
MAJORITY CGGAAGGGGCCTTCGTGGGCTTTGTCCTTTCCCGCCCCGAGCCCATGTGGGCCGAGCTTCTGGCCCTGGC
DNAPTAQ .....G.....AAG.....T..... 974
DNAPTFL .....T..TT.....TC.T.....T..... 971
DNAPTTH .....C.....C.....G.....AAA..... 980
MAJORITY CGCCGCCAGGGAGGGCCGGGTCCACCGGGCACCAGACCCCTTTAXGGGCCTXAGGGACCTXAAGGAGGTG
DNAPTAQ .....G.....C..C..G..T.A..AA.C...C.....G.....C. 1044
DNAPTFL T.GG..GT.....G..CC....T.....A.....C...G.....G.....T....G.... 1041
DNAPTTH ....TG.....C.....G.....G.....GGC...G..A.A.....C.....C 1050

# FIG. 2D

```
MAJORITY [SEQ ID NO:7] CGGGGXCTCCTCGCCAAGGACCTGGCCGTTTTGGCCCTGAGGGAGGGCCTXGACCTCXTGCCCGGGGACG

DNAPTAQ  [SEQ ID NO:1] .....G..T........A......AG....C.............A.....T.G....CC......C.... 1114
DNAPTFL  [SEQ ID NO:2] .....AA....G.............G.............C..........G......T.C..A.A..... 1111
DNAPTTH  [SEQ ID NO:3] .....C..................C.....C......TC.........G..A......G.......... 1120

MAJORITY ACCCCATGCTCCTCGCCTACCTCCTGGACCCCTCGAACACCACCCCCGAGGGGCTGGCCCGGCGCTACGG

DNAPTAQ  ..................................T..................................... 1184
DNAPTFL  ............G........T.........................T..................T..... 1181
DNAPTTH  ..................................................G.......... 1190

MAJORITY GGGGGAGTGGACGGAGGAXGCGGGGGAGCGGGCCCTCCTXTCGGAGAGGCTCTTCCXGAACCTXXXGGAG

DNAPTAQ  C..................G..............GC...T..............GCC.....GTG..G. 1254
DNAPTFL  ...................T.........A.........GG.....C.C.......A..C...AAA.... 1251
DNAPTTH  ...................C..C.CCC.C..........C..G.........CAT.G......CCTTA.. 1260

MAJORITY CGCCTTGAGGGGGAGGAGAGGCTCCTTTGGCTTTACCAGGAGGTGGAGAAGCCCCTTTCCCGGGTCCTGG

DNAPTAQ  A.G.....................................G..........G..........GCT....... 1324
DNAPTFL  .....A....A..A..AC.C..G.............G..................G...........GT... 1321
DNAPTTH  .....C.............A.............C.....C........A........C.............. 1330

MAJORITY CCCACATGGAGGCCACGGGGGTXCGGCTGGACGTGGCCTACCTCCAGGCCCTXTCCCTGGAGGTGGCGGA

DNAPTAQ  .....................G..C.............T...AG....T.G..............C.. 1394
DNAPTFL  ...GG.................C.....................................C...A..C 1391
DNAPTTH  ................C.....A.....................T........T.........C.T..... 1400
```

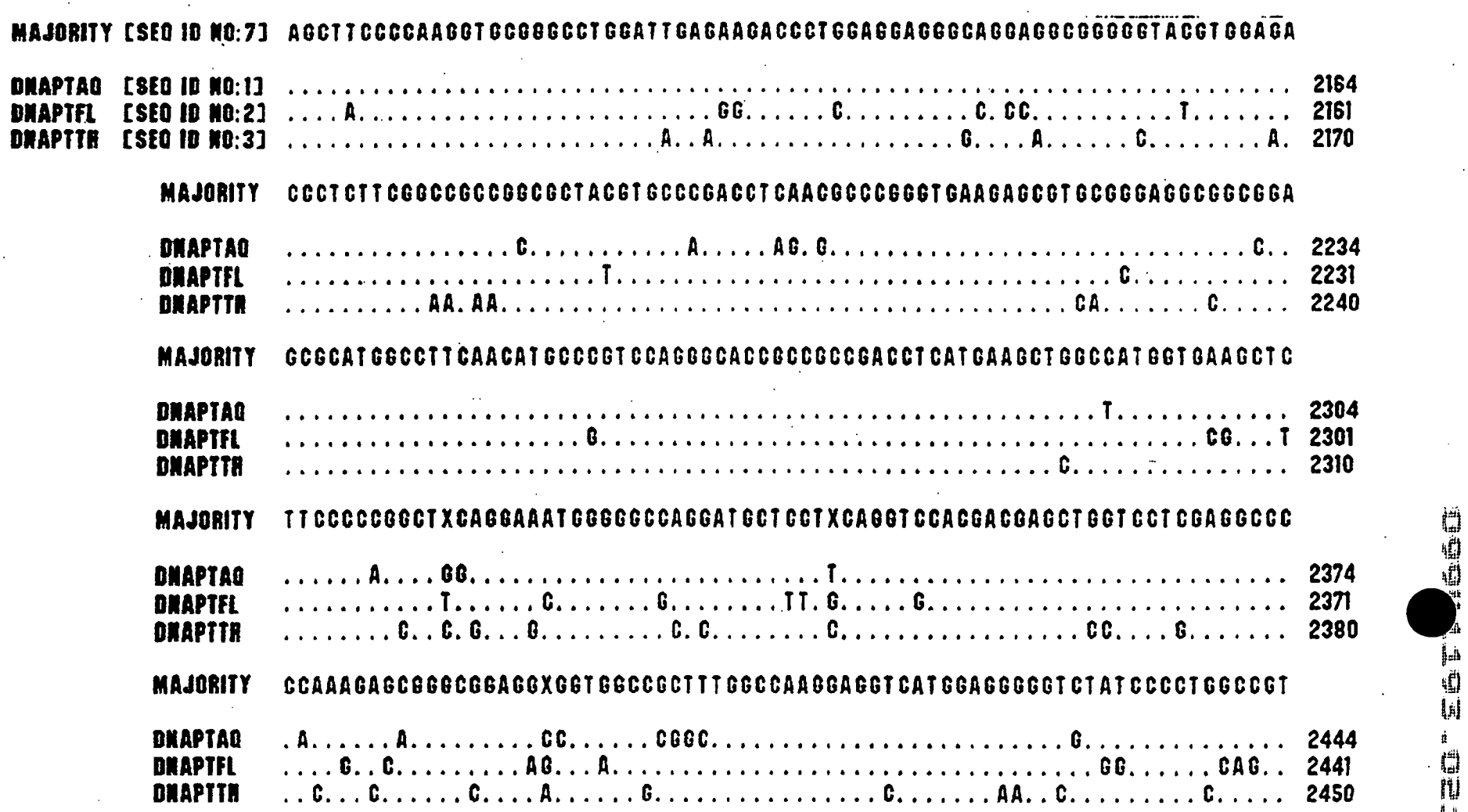
# FIG. 2E

```
MAJORITY [SEQ ID NO:7] GGAGATCCGCCGCCTCGAGGAGGAGGTCTTCGGCCTGGCCGGCCACCCCTTCAACCTCAACTCCCGGGAC

DNAPTAQ  [SEQ ID NO:1] .......GC..........CC.....................................................  1464
DNAPTFL  [SEQ ID NO:2] ....G.G....AG..G.....................................................C...  1461
DNAPTTH  [SEQ ID NO:3] ..................................T....G.....................................  1470

MAJORITY CAGCTGGAAAGGGTGCTCTTTGACGAGCTXGGGCTTCCCGCCATCGGCAAGACGGAGAAGACXGGCAAGC

DNAPTAQ  ..............C..............A.....................................C.......  1534
DNAPTFL  ........GC.....................G..C..G..T.....................G..G..A.  1531
DNAPTTH  ..............................TA..........T.G..G......C.A.....A.......  1540

MAJORITY GCTCCACCAGCGCCGCCGTGCTGGAGGCCCTXCGXGAGGCCCACCCCATCGTGGAGAAGATCCTGCAGTA

DNAPTAQ  ....................C..........C..C.....................................  1604
DNAPTFL  .............T.....................G..A.....................CCGC.........  1601
DNAPTTH  ................G..............A..G.....................................C...C.  1610

MAJORITY CCGGGAGCTCACCAAGCTCAAGAACACCTACATXGACCCCCTGCCXGXCCTCGTCCACCCCAGGACGGGC

DNAPTAQ  ..................G....G.........T......T....G.A....A.....................  1674
DNAPTFL  ..................................A..........C.C...G..........A...C...  1671
DNAPTTH  ..............................G.G........C..AAG............G.........  1680

MAJORITY CGCCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGGCAGGCTTAGTAGCTCCGACCCCAACCTGC

DNAPTAQ  ..................................................A..........T........C.  1744
DNAPTFL  ..G..............................C..............TCC.....................  1741
DNAPTTH  ..................................................G.....................  1750
```

# FIG. 2F

```
MAJORITY [SEQ ID NO:7] AGAACATCCCCGTCCGCACCCCXCTGGGCCAGAGGATCCGCCGGGCCTTCGTGGCCGAGGAGGGXTGGGT

DNAPTAQ  [SEQ ID NO:1] ......................G..T..G.....................A.C..........G...C.  1814
DNAPTFL  [SEQ ID NO:2] ............G.........T.........C.C.......A.....................C.....  1811
DNAPTTH  [SEQ ID NO:3] ......................CT.....................................C...T....C 1820

MAJORITY GTTGGTGGCCCTGGACTATAGCCAGATAGAGCTCCGGGTCCTGGCCCACCTCTCCGGGGACGAGAACCTG

DNAPTAQ  A.....................................A....G.................C.............  1884
DNAPTFL  .C......T.T.......C.........T.....T.....................................  1881
DNAPTTH  .....................................C.....C.....................A......  1890

MAJORITY ATCCGGGTCTTCCAGGAGGGGAGGGACATCCACACCCAGACCGCCAGCTGGATGTTCGGCGTCCCCCCGG

DNAPTAQ  ......................C.............GG.................................G..  1954
DNAPTFL  ............T.....................................................TT....C.  1951
DNAPTTH  ...A..................A.....................A.............................  1960

MAJORITY AGGCCGTGGACCCCCTGATGCGCCGGGCGGCCAAGACCATCAACTTCGGGGTCCTCTACGGCATGTCCGC

DNAPTAQ  .....................................................................G..  2024
DNAPTFL  .A.GG..A.....T.....................................G.................  2021
DNAPTTH  ..................................GG.G........C.....................  2030

MAJORITY CCACCGCCTCTCCCAGGAGCTTGCCATCCCCTACGAGGAGGCGGTGGCCTTCATTGAGCGCTACTTCCAG

DNAPTAQ  ......................A........T...........CCA.....................T...  2094
DNAPTFL  ............GG.......T.....................................................  2091
DNAPTTH  ...TA.G.....................................T..A.............A  2100
```

# FIG. 2G


MAJORITY [SEQ ID NO:7] AGCTTCCCCAAGGTGCGGGCCTGGATTGAGAAGACCCTGGAGGAGGGCAGGAGGCGGGGGTACGTGGAGA
DNAPTAQ [SEQ ID NO:1] 2164
DNAPTFL [SEQ ID NO:2] 2161
DNAPTTH [SEQ ID NO:3] 2170
MAJORITY CCCTCTTCGGCCGCCGGCGCTACGTGCCCGACCTCAACGCCCGGGTGAAGAGCGTGCGGGAGGCGGCGGA
DNAPTAQ 2234
DNAPTFL 2231
DNAPTTH 2240
MAJORITY GCGCATGGCCTTCAACATGCCCGTCCAGGGCACCGCCGCCGACCTCATGAAGCTGGCCATGGTGAAGCTC
DNAPTAQ 2304
DNAPTFL 2301
DNAPTTH 2310
MAJORITY TTCCCCCGGCTXCAGGAAATGGGGGCCAGGATGCTGCTXCAGGTCCACGACGAGCTGGTCCTCGAGGCCC
DNAPTAQ 2374
DNAPTFL 2371
DNAPTTH 2380
MAJORITY CCAAAGAGCGGGCGGAGGXGGTGGCCGCTTTGGCCAAGGAGGTCATGGAGGGGTCTATCCCCTGGCCGT
DNAPTAQ 2444
DNAPTFL 2441
DNAPTTH 2450

# FIG. 2H

```
MAJORITY [SEQ ID NO:7]  GCCCCTGGAGGTGGAGGTGGGGATGGGGGAGGACTGGCTCTCCGCCAAGGAGTAG

DNAPTAQ  [SEQ ID NO:1]  .....................A.....................GA 2499
DNAPTFL  [SEQ ID NO:2]  ...................CC..................... 2496
DNAPTTH  [SEQ ID NO:3]  .....................T.....GT... 2505
```

# FIG. 3A

```
MAJORITY [SEQ ID NO:8] MXAML PLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEPVQAVYGFAKSLLKALKEDG· DAVXVVFDAK

TAQ PRO  [SEQ ID NO:4] .RG.....H.....I..... 69
TFL PRO  [SEQ ID NO:5] .-.....V.V..... 68
TTH PRO  [SEQ ID NO:6] .E.....YK..F..... 70

MAJORITY APSFRHEAYEAYKAGRAPTPEDFPRQLALI KELVDLLGLXRLEVPGYEADDVLATLAKKAEKEGYEVRI L

TAQ PRO  .....GG.....A.....S..... 139
TFL PRO  .....V.....F.....R..... 138
TTH PRO  .....FT..... 140

MAJORITY TADRDLYQLLSDRI AVLHPEGYLI TPAWLWEKYGLRPEQWVDYRALXGDPSDNLPGVKGI GEKTAXKLLX

TAQ PRO  ...K.....H.....D..A.....T..E.....R...E 209
TFL PRO  .....E...I.....Y.....A.....I.....QR.I R 208
TTH PRO  .....V...V.....H....E.....F...V.....L...K 210

MAJORITY EWGSLENLLKNLDRVKP· XXREKI XAHMEDLXLSXXLSXVRTDLPLEVDFAXRREPDREGLRAFLERLEF

TAQ PRO  .....A.....L..·AI....L...D..K..WD.AK.....K.....R..... 278
TFL PRO  .....FQH..Q...·SL...LQ.G..A.A..RK..Q.H.....GR..T.NL..... 277
TTH PRO  .....ENV....K..L...R..LE..R.....L.QG..... 280

MAJORITY GSLLHEFGLLEXPKALEEAPWPPPEGAFVGFVLSRPEPMWAELLALAAARXGRVHRAXDPLXGLRDLKEV

TAQ PRO  .....S.....K.....D.....G.....PE.YKA.....A 348
TFL PBO  .....G...A.....L..SF.....G.WE..L...Q...R.....G. 347
TTH PRO  .....A.AP.....K....C.D.....A...A..K..... 350
```

# FIG. 3B

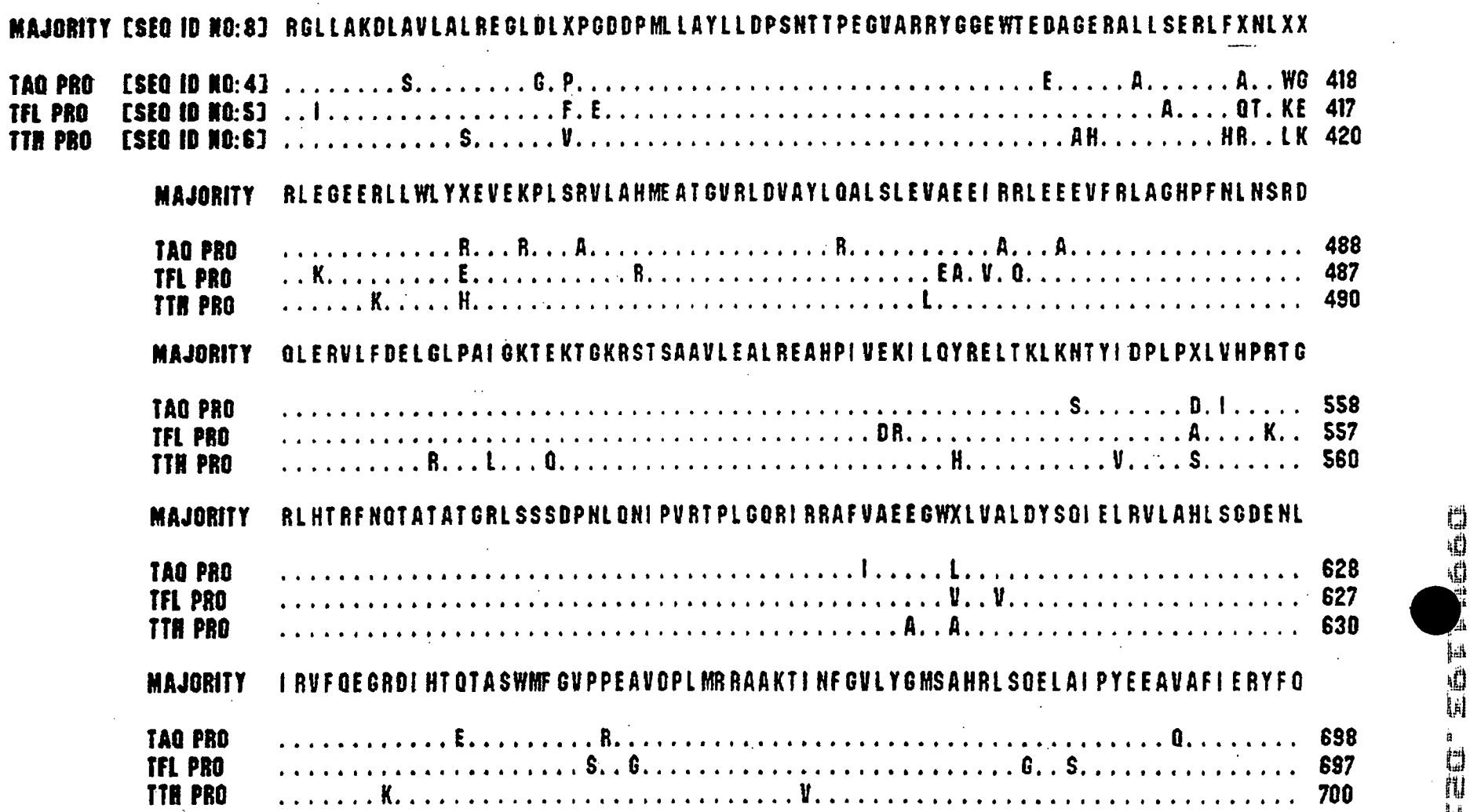
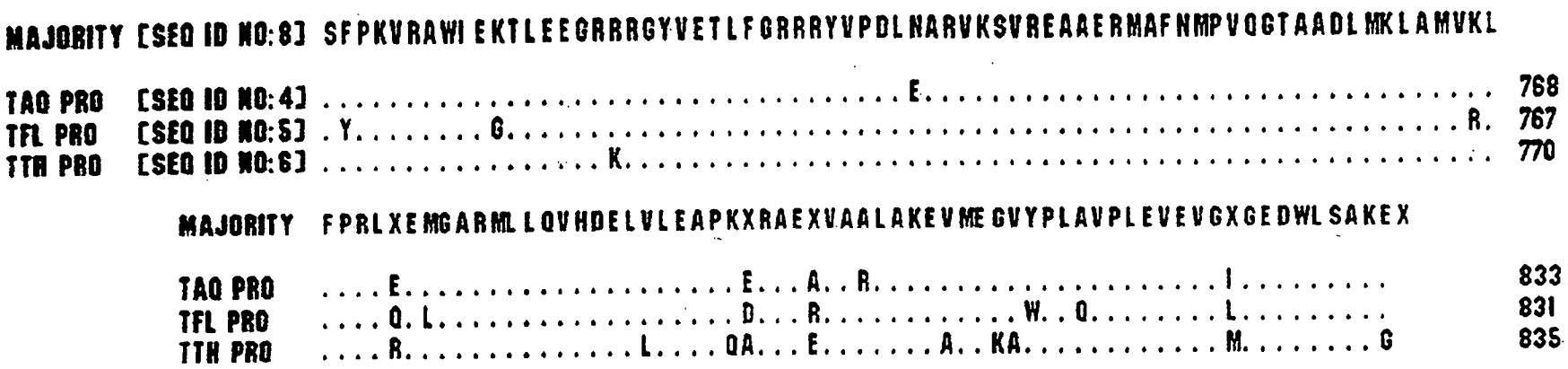

MAJORITY [SEQ ID NO:8] RGLLAKDLAVLALREGLDLXPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEDAGERALLSERLFXNLXX
TAQ PRO [SEQ ID NO:4] .....S.....G.P.....E.....A.....A..WG 418
TFL PRO [SEQ ID NO:5] ..I.....F.E.....A....QT.KE 417
TTH PRO [SEQ ID NO:6] .....S.....V.....AH.....HR..LK 420
MAJORITY RLEGEERLLWLYXEVEKPLSRVLAHMEATGVRLDVAYLQALSLEVAEEIRRLEEEVFRLAGHPFNLNSRD
TAQ PRO .....R...R...A.....R.....A...A..... 488
TFL PRO ..K.....E.....R.....EA.V.Q..... 487
TTH PRO .....K.....H.....L..... 490
MAJORITY QLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKNTYIDPLPXLVHPRTG
TAQ PRO .....S.....D.I..... 558
TFL PRO .....DR.....A....K.. 557
TTH PRO .....R...L...Q.....H.....V....S..... 560
MAJORITY RLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFVAEEGWXLVALDYSQIELRVLAHLSGDENL
TAQ PRO .....I.....L..... 628
TFL PRO .....V..V..... 627
TTH PRO .....A..A..... 630
MAJORITY IRVFQEGRDIHTQTASWMFGVPPEAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIPYEEAVAFIERYFQ
TAQ PRO .....E.....R.....Q..... 698
TFL PRO .....S..G.....G..S..... 697
TTH PRO .....K.....V..... 700

FIG. 3C


MAJORITY [SEQ ID NO:8] SFPKVRAWIEKTLEEGRRRGYVETLFGRRRYVPDLNARVKSVREAAERMAFNMPVQGTAADLMKLAMVKL
TAQ PRO [SEQ ID NO:4] .....E..... 768
TFL PRO [SEQ ID NO:5] .Y.....G.....R. 767
TTH PRO [SEQ ID NO:6] .....K..... 770
MAJORITY FPRLXEMGARMLLQVHDELVLEAPKXRAEXVAALAKEVMEGVYPLAVPLEVEVGXGEDWLSAKEX
TAQ PRO ....E.....E...A..R.....I..... 833
TFL PRO ....Q.L.....D...R.....W..Q.....L..... 831
TTH PRO ....R.....L....QA...E.....A..KA.....M.....G 835

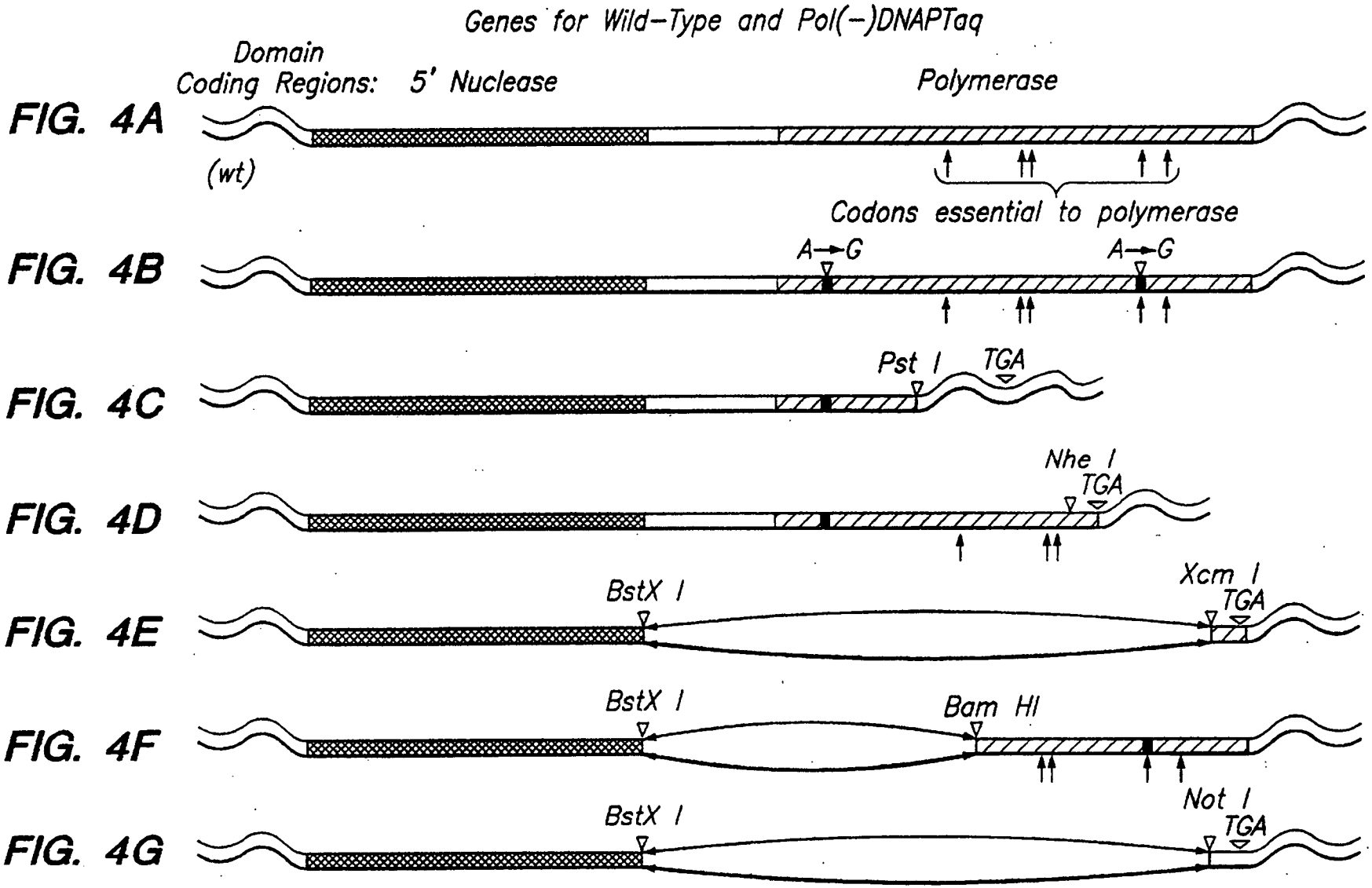



FIG. 4A

FIG. 4B

FIG. 4C

FIG. 4D

FIG. 4E

FIG. 4F

FIG. 4G

Genes for Wild-Type and Pol(-) DNAPTfl

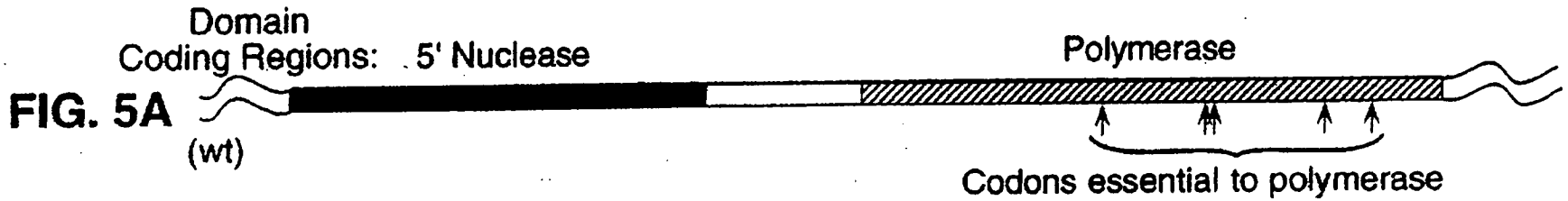



FIG. 5A


FIG. 5B

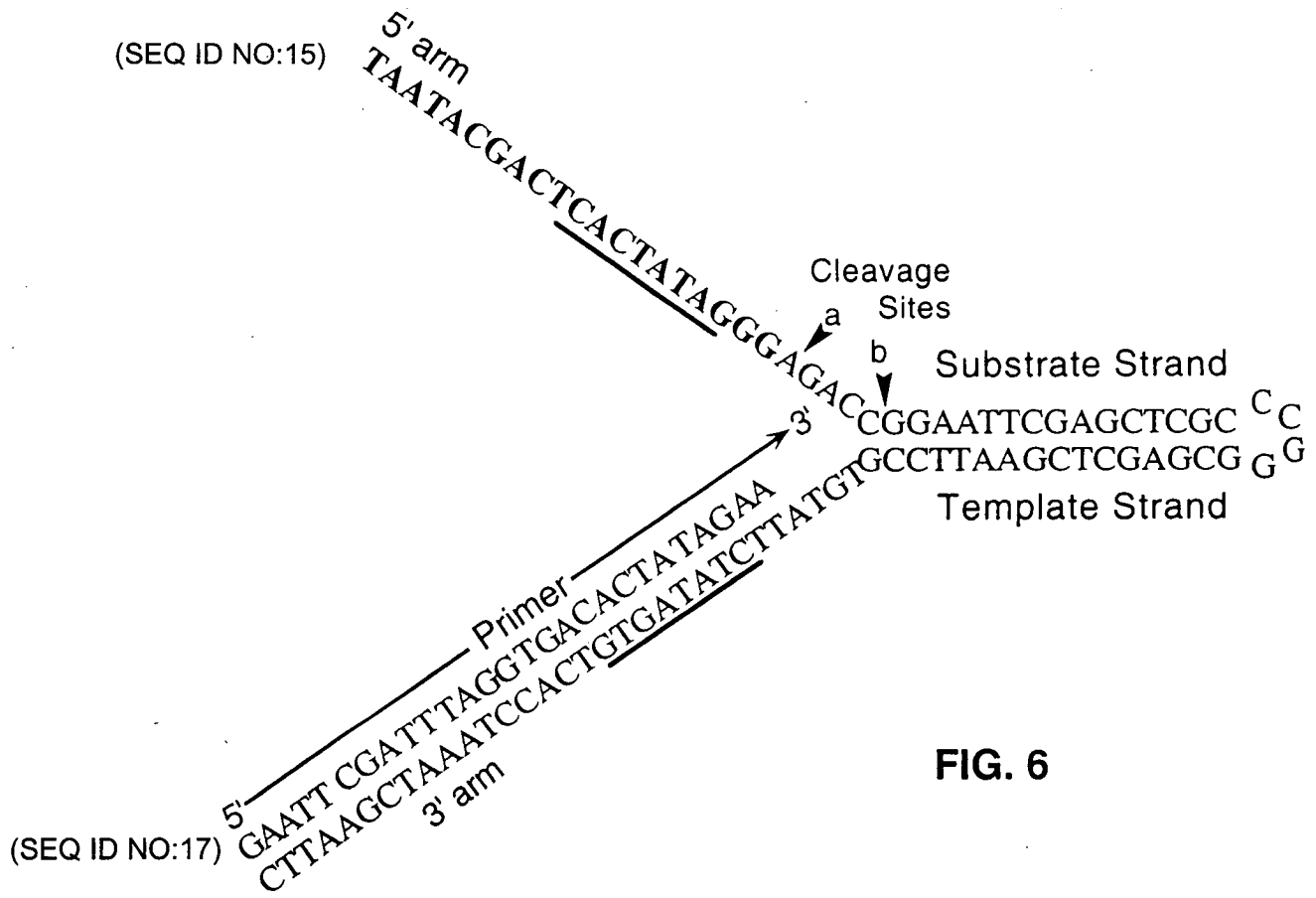

(SEQ ID NO:15)
5' arm
TAATACGACTCACTATAGGGAGAC
Cleavage
Sites
a
b
Substrate Strand
3'
CGGAATTCGAGCTCGC C C
GCCTTAAGCTCGAGCG G G
Template Strand
Primer
5'
GAATT CGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTATGT
3' arm
(SEQ ID NO:17)

**FIG. 6**

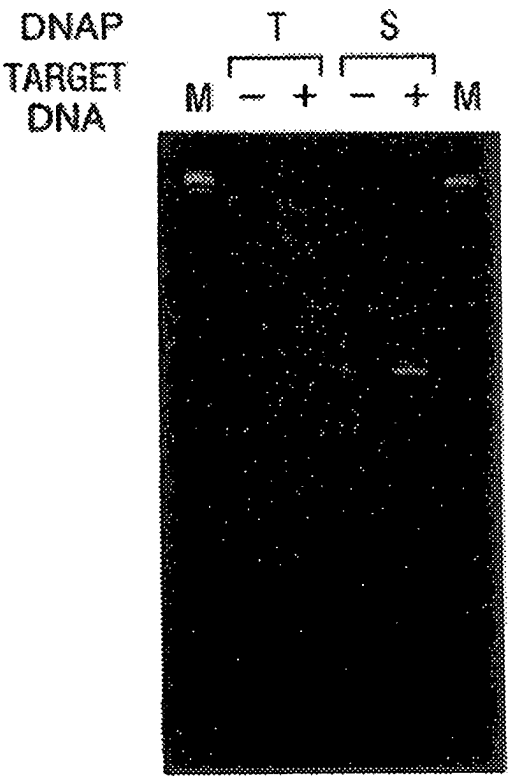



FIG. 7

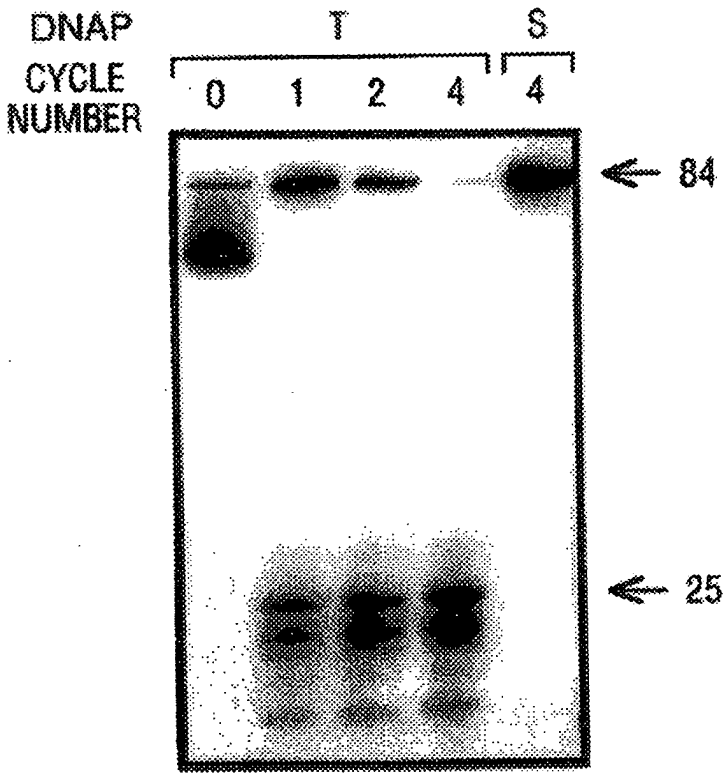



FIG. 8

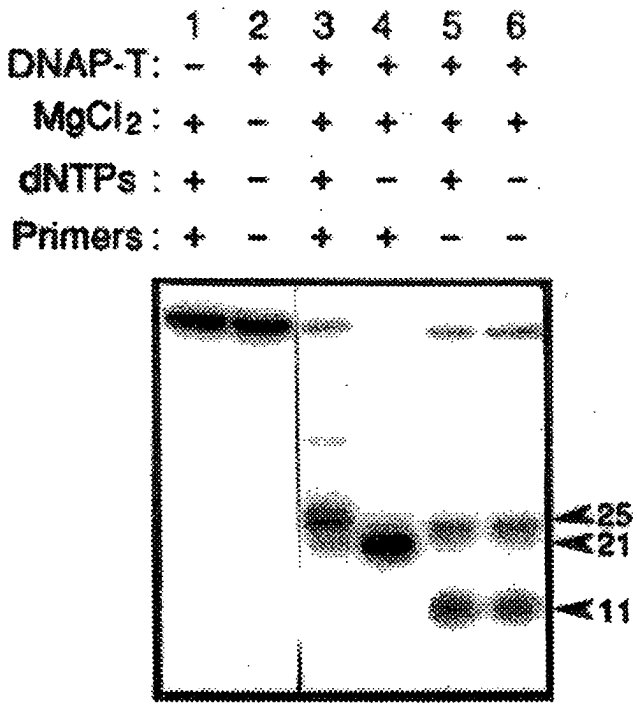



FIG. 9A

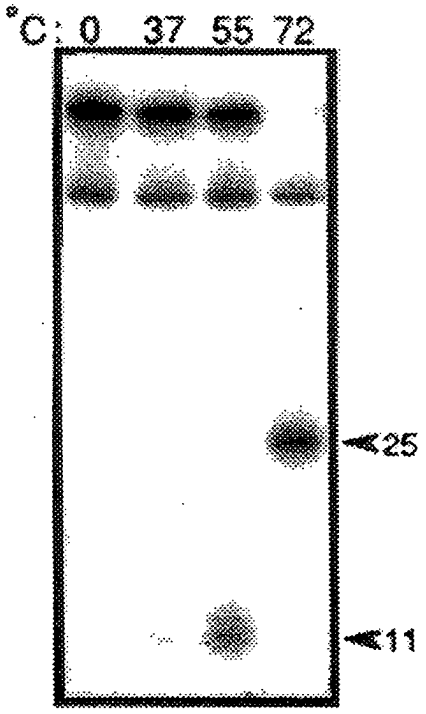



FIG. 9B

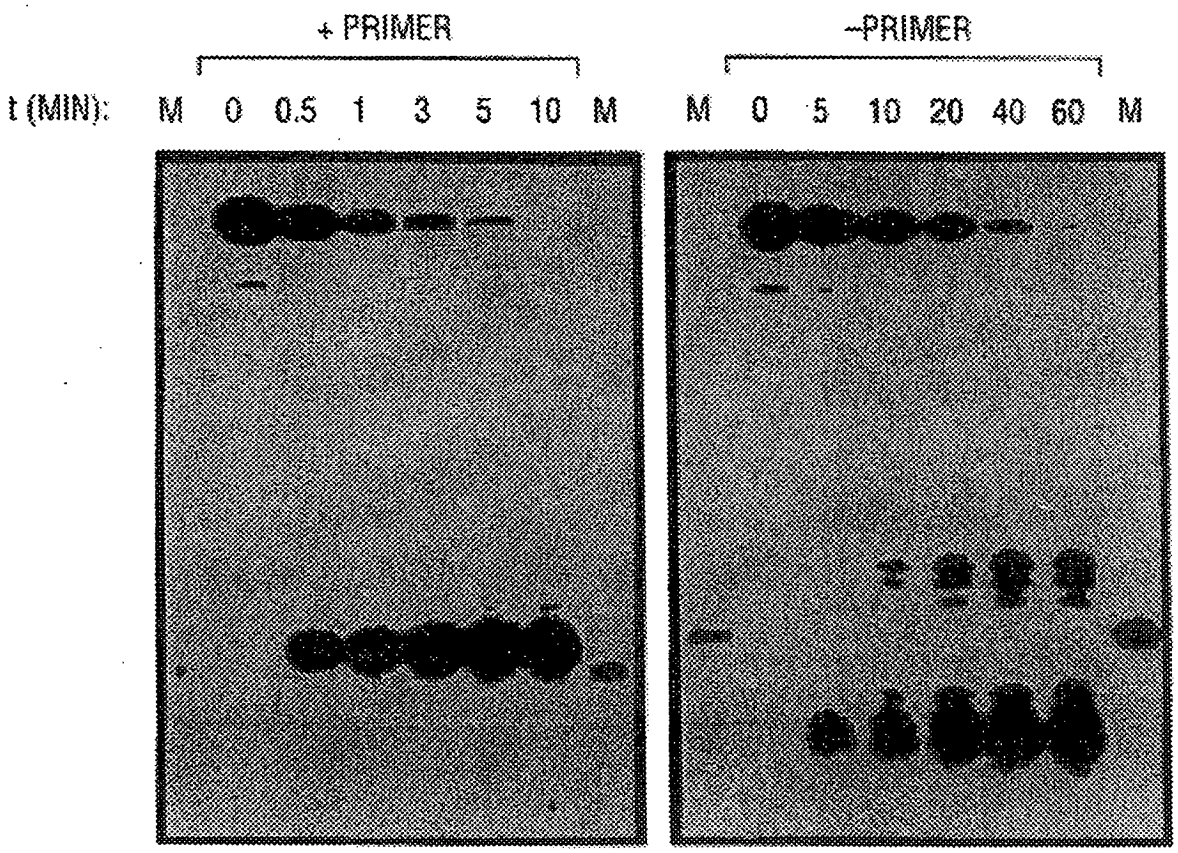



FIG. 10A

FIG. 10B

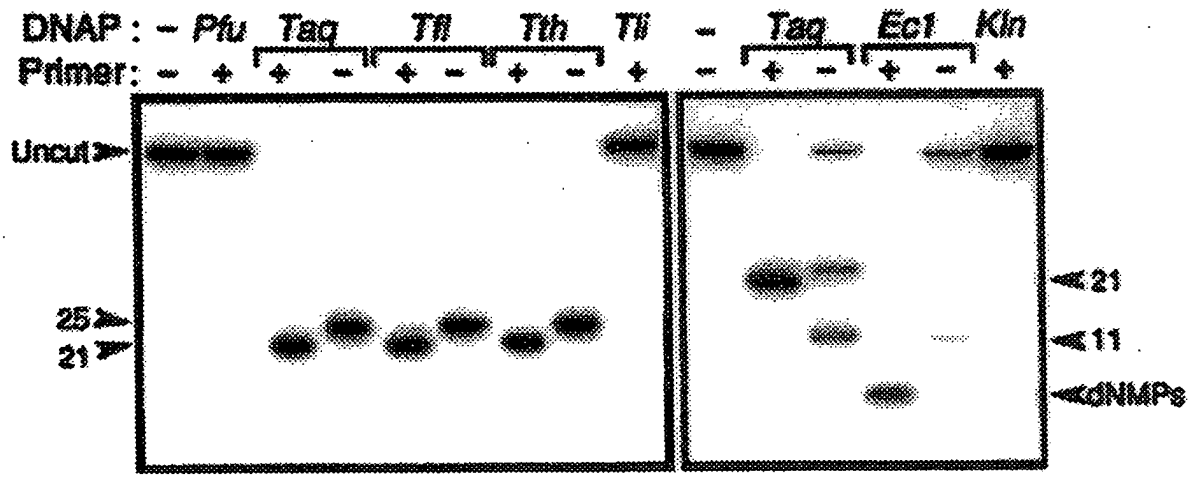
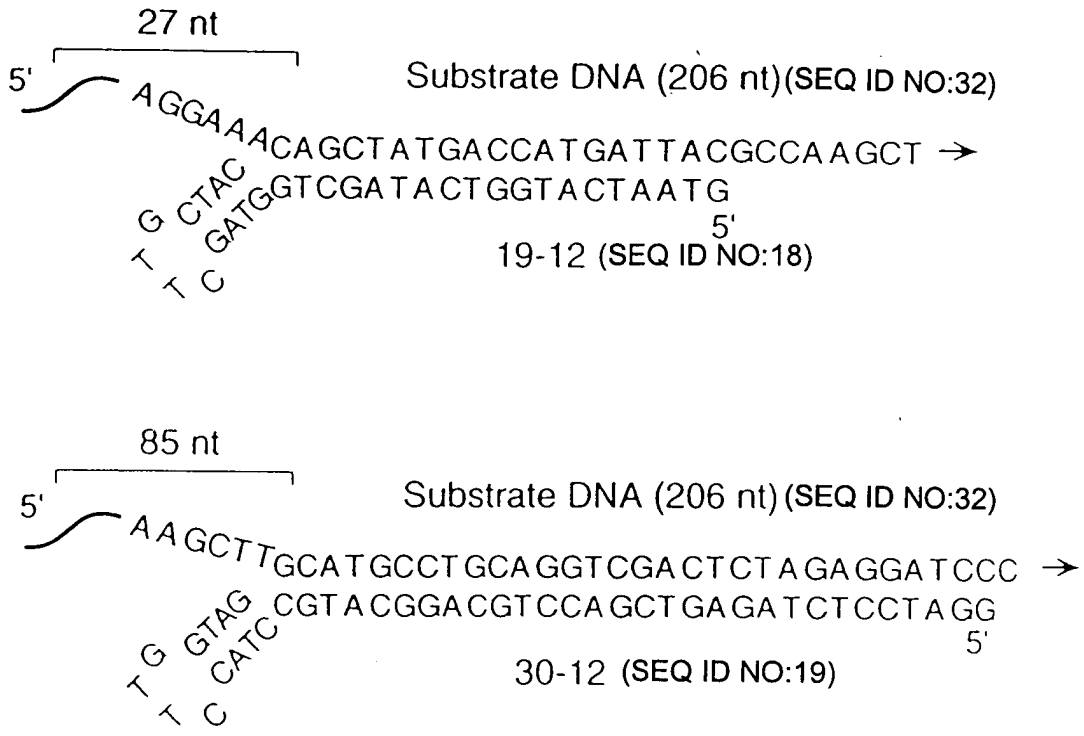



FIG. 11A

FIG. 11B

# FIG. 12A


27 nt
5'
Substrate DNA (206 nt) (SEQ ID NO:32)
AGGAAACAGCTATGACCATGATTACGCCAAGCT →
TG CTAC
T GATGGTCGATACTGGTACTAATG
T C
5'
19-12 (SEQ ID NO:18)
85 nt
5'
Substrate DNA (206 nt) (SEQ ID NO:32)
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCC →
TG GTAG CGTACGGACGTCCAGCTGAGATCTCCTAGG
T CATC
5'
30-12 (SEQ ID NO:19)

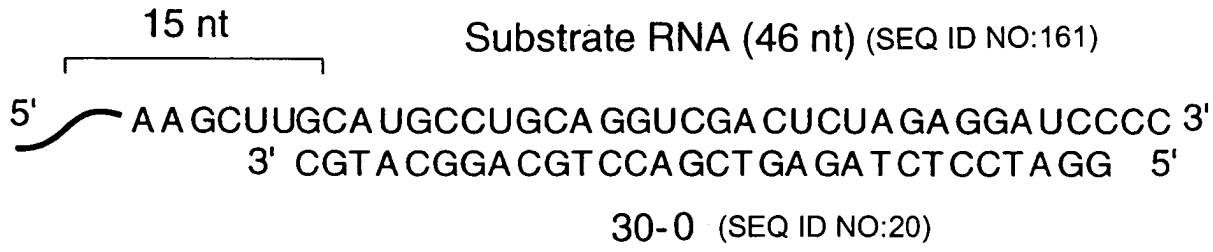

15 nt
Substrate RNA (46 nt) (SEQ ID NO:161)
5'
AAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCC 3'
3' CGTACGGACGTCCAGCTGAGATCTCCTAGG 5'
30-0 (SEQ ID NO:20)

FIG. 13A

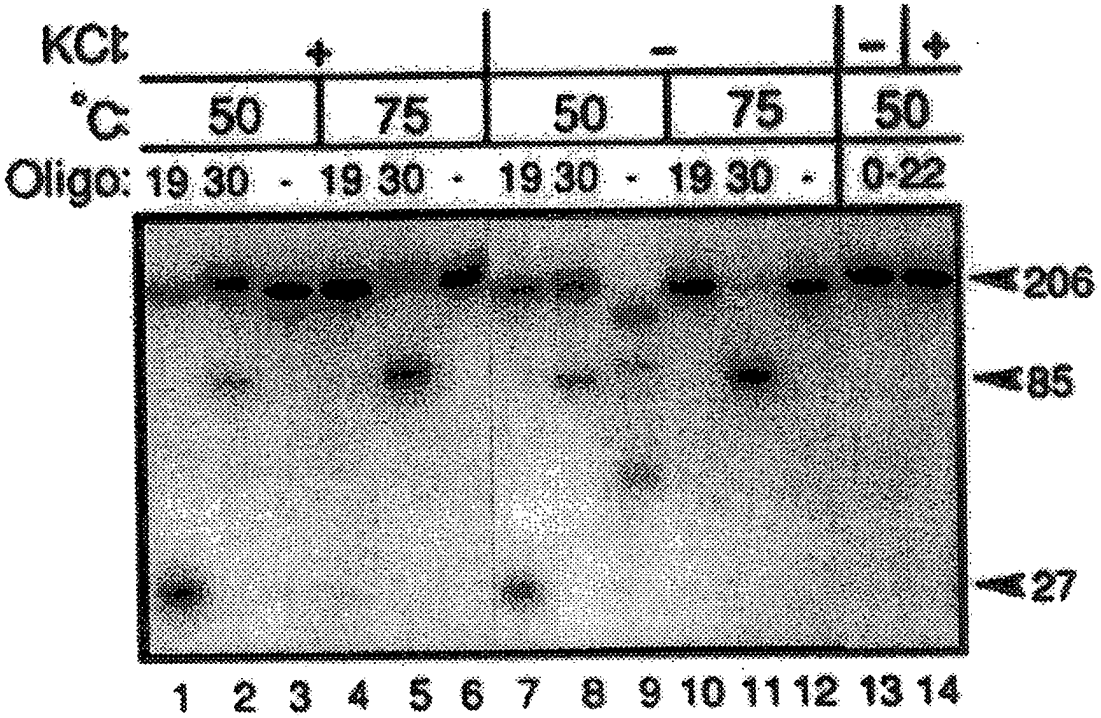



FIG. 12B


FIG. 13B

(SEQ ID NO:162)

-35 -10 RBS

TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG

MetAsnSer...

ATGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCC

EcoRI SstI KpnI SmaI BamHI XbaI SalI PstI SphI HindIII

FIG. 14B

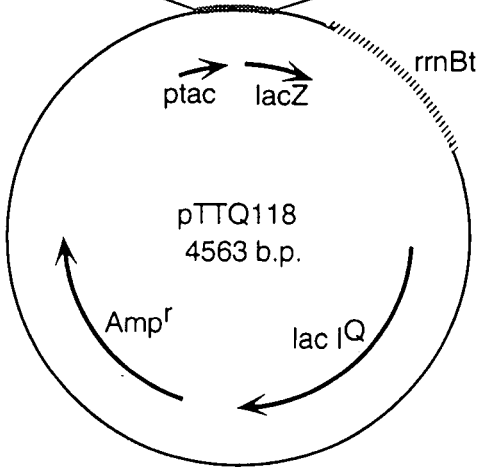



FIG. 14A

FIG. 14C

RBS: Ribosome binding site
ptac: Synthetic tac promoter
lac $I^Q$: Lac repressor gene

lacZ: Beta-galactosidase alpha fragment
rrnBt: E. coli rrnB transcription terminator

(SEQ ID NO:163)

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTT

Bgl II — T7 Promoter — XbaI

MetAlaSer...

AACTTTAAGAAGGAGATATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGATCCGGCT

RBS — NdeI — BamHI

FIG. 15B

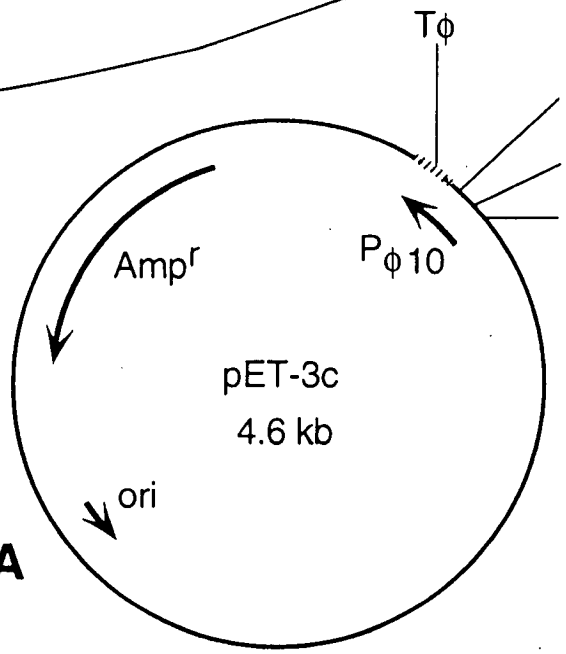



FIG. 15A

$P_{\phi 10}$: Bacteriophage T7 ϕ10 promoter

RBS: Ribosome binding site

Tϕ: T7 ϕ Terminator

FIG. 15C

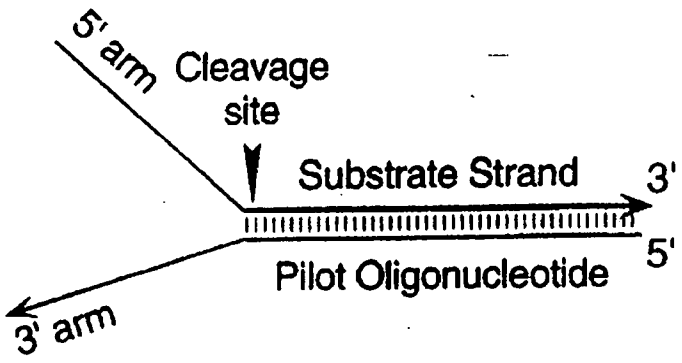



FIG. 16A

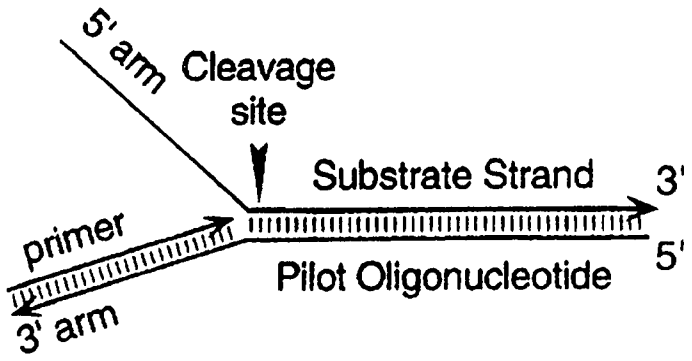



FIG. 16B

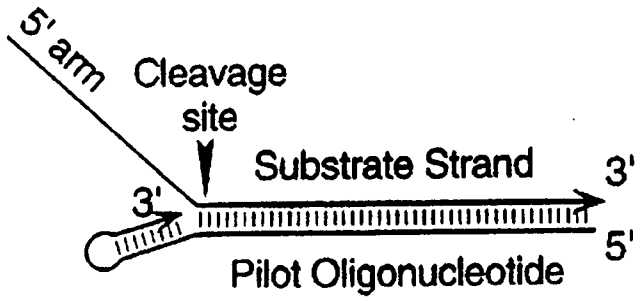



FIG. 16C

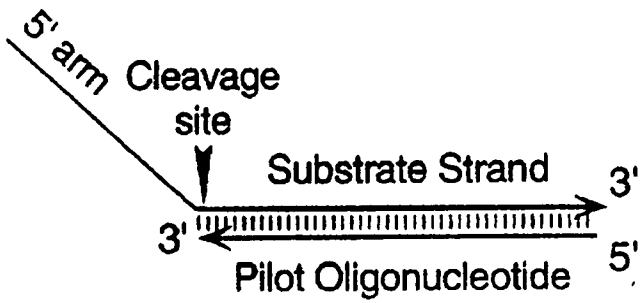



FIG. 16D

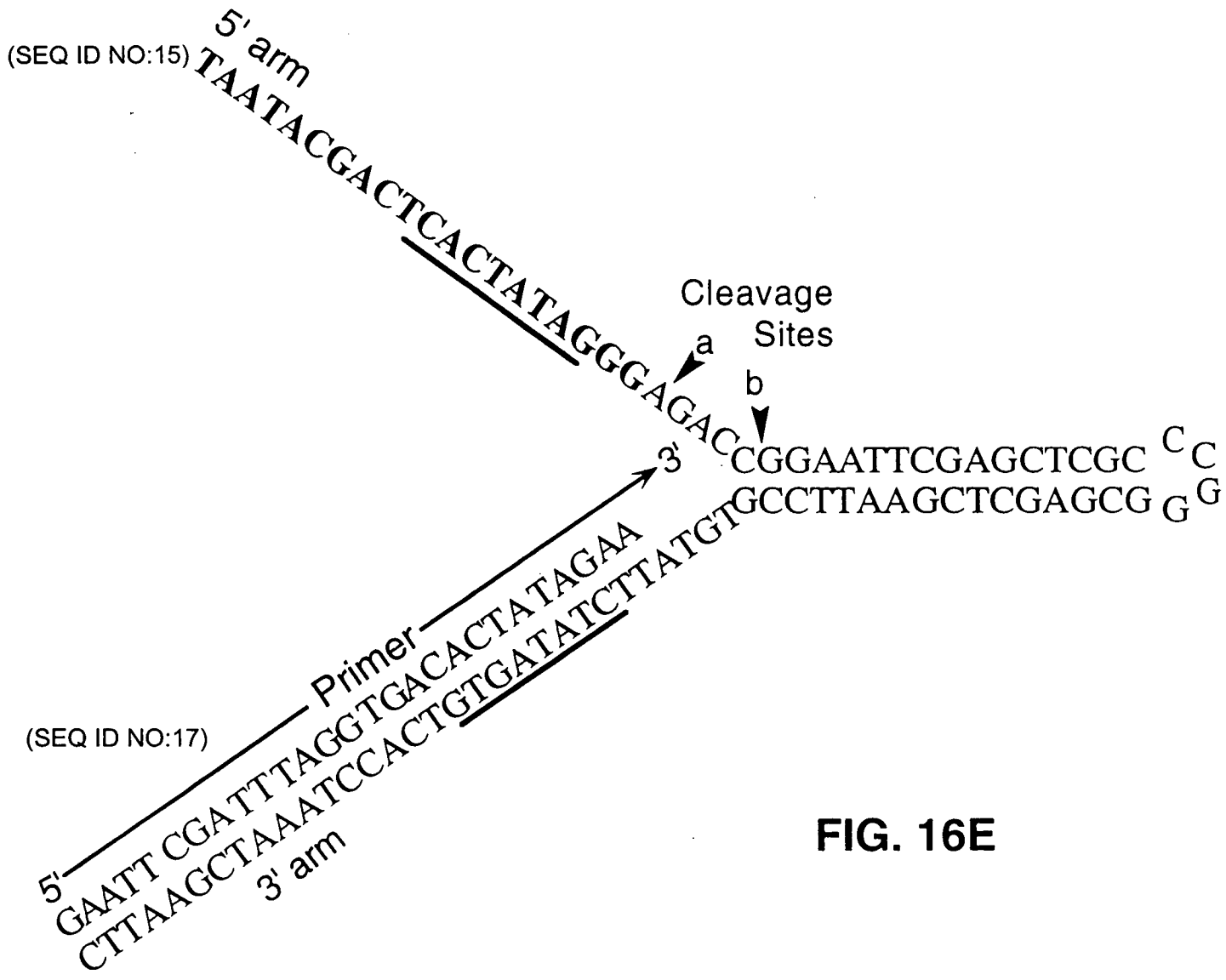

(SEQ ID NO:15)
5' arm
TAATACGACTCACTATAGGGAGAC
Cleavage
Sites
a
b
3'
CGGAATTCGAGCTCGC C C
GCCTTAAGCTCGAGCG G G
Primer
(SEQ ID NO:17)
5'
GAATT CGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTATGT
3' arm

FIG. 16E

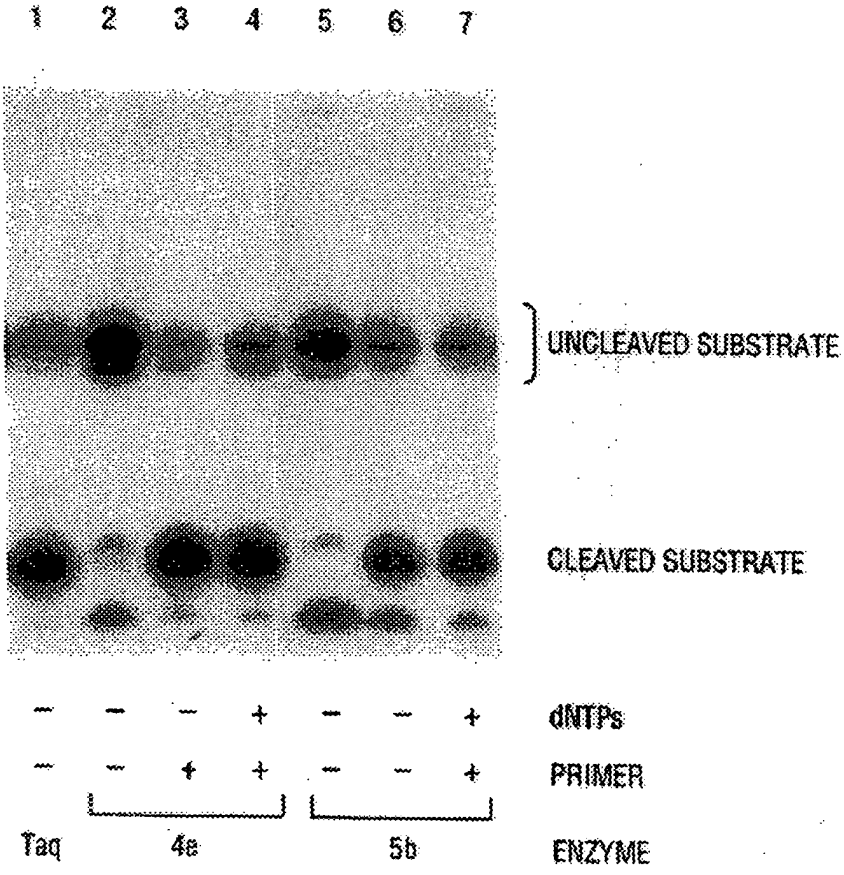

1 2 3 4 5 6 7
UNCLEAVED SUBSTRATE
CLEAVED SUBSTRATE
– – – + – – + dNTPs
– – + + – – + PRIMER
Taq 4e 5b ENZYME

FIG. 17

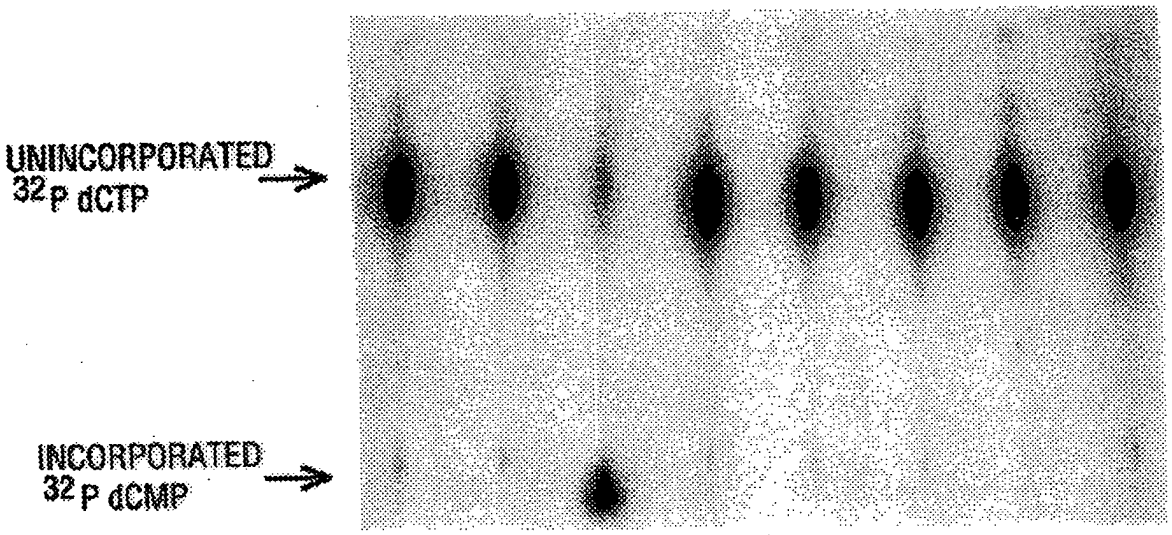

UNINCORPORATED
32P dCTP
INCORPORATED
32P dCMP
ENZYME Taq – Taq 4b 4c 4d 4e 4f
PRIMED M13 – + + + + + + +

FIG. 18

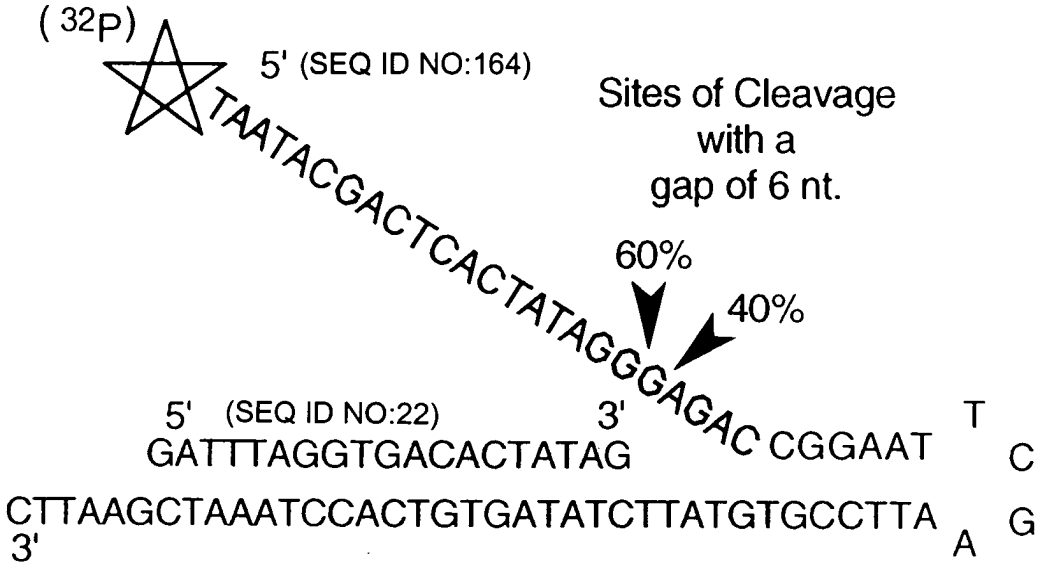

( 32P)
5' (SEQ ID NO:164)
TAATACGACTCACTATAGGGAGAC
Sites of Cleavage
with a
gap of 6 nt.
60%
40%
5' (SEQ ID NO:22)
3'
GATTTAGGTGACACTATAG
CGGAAT
T
C
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA
A
G
3'

**FIG. 19A**

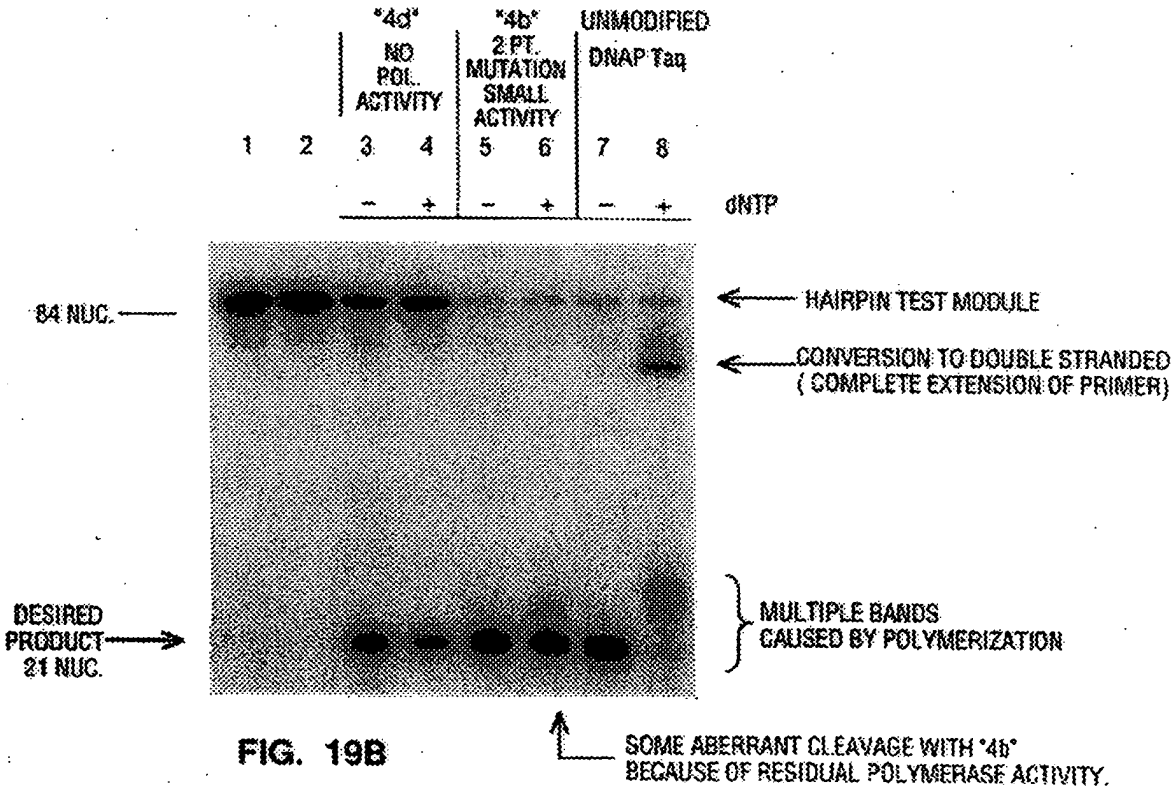

"4d"
NO POL. ACTIVITY
"4b"
2 PT. MUTATION SMALL ACTIVITY
UNMODIFIED
DNAP Taq
1 2 3 4 5 6 7 8
- + - + - +
dNTP
84 NUC.
HAIRPIN TEST MODULE
CONVERSION TO DOUBLE STRANDED
( COMPLETE EXTENSION OF PRIMER)
DESIRED PRODUCT
21 NUC.
MULTIPLE BANDS
CAUSED BY POLYMERIZATION
SOME ABERRANT CLEAVAGE WITH "4b"
BECAUSE OF RESIDUAL POLYMERASE ACTIVITY.

FIG. 19B

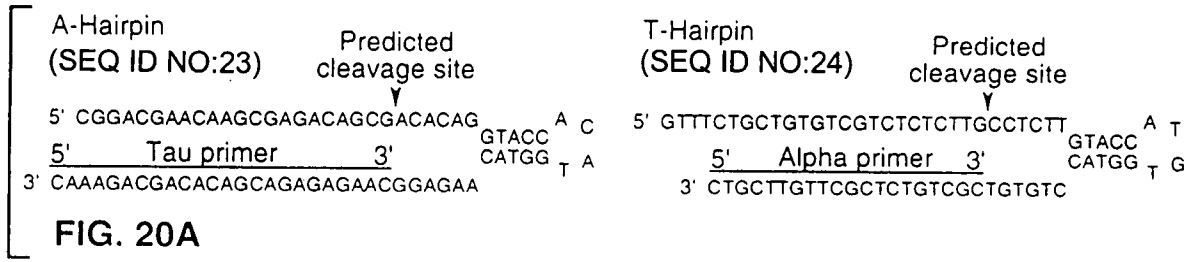



FIG. 20A

Sequence of alpha primer: (SEQ ID NO:25)

5' GACGAACAAGCGAGACAGCG 3'

FIG. 20B

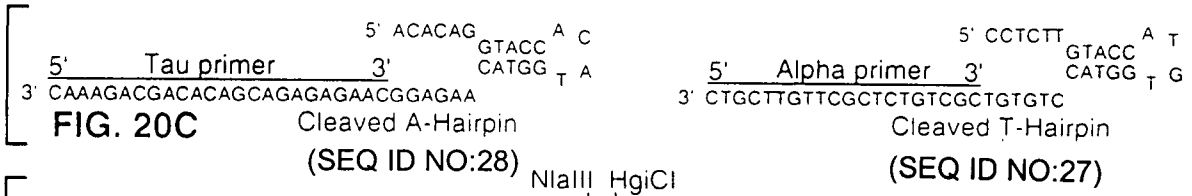



FIG. 20C

Cleaved A-Hairpin
(SEQ ID NO:28)

Cleaved T-Hairpin
(SEQ ID NO:27)

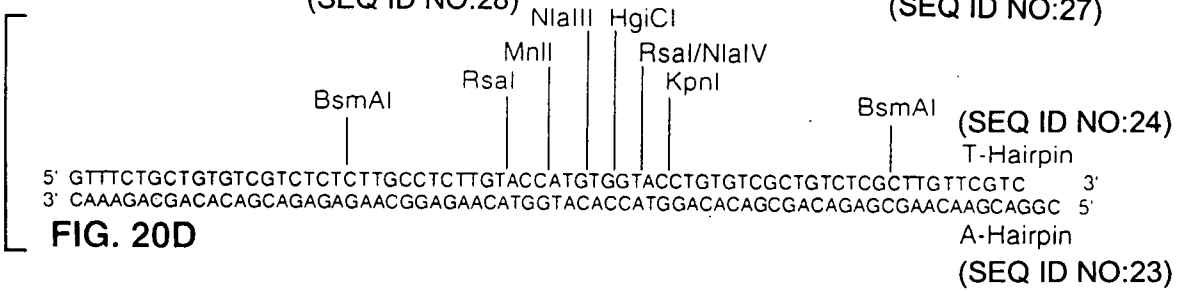



FIG. 20D

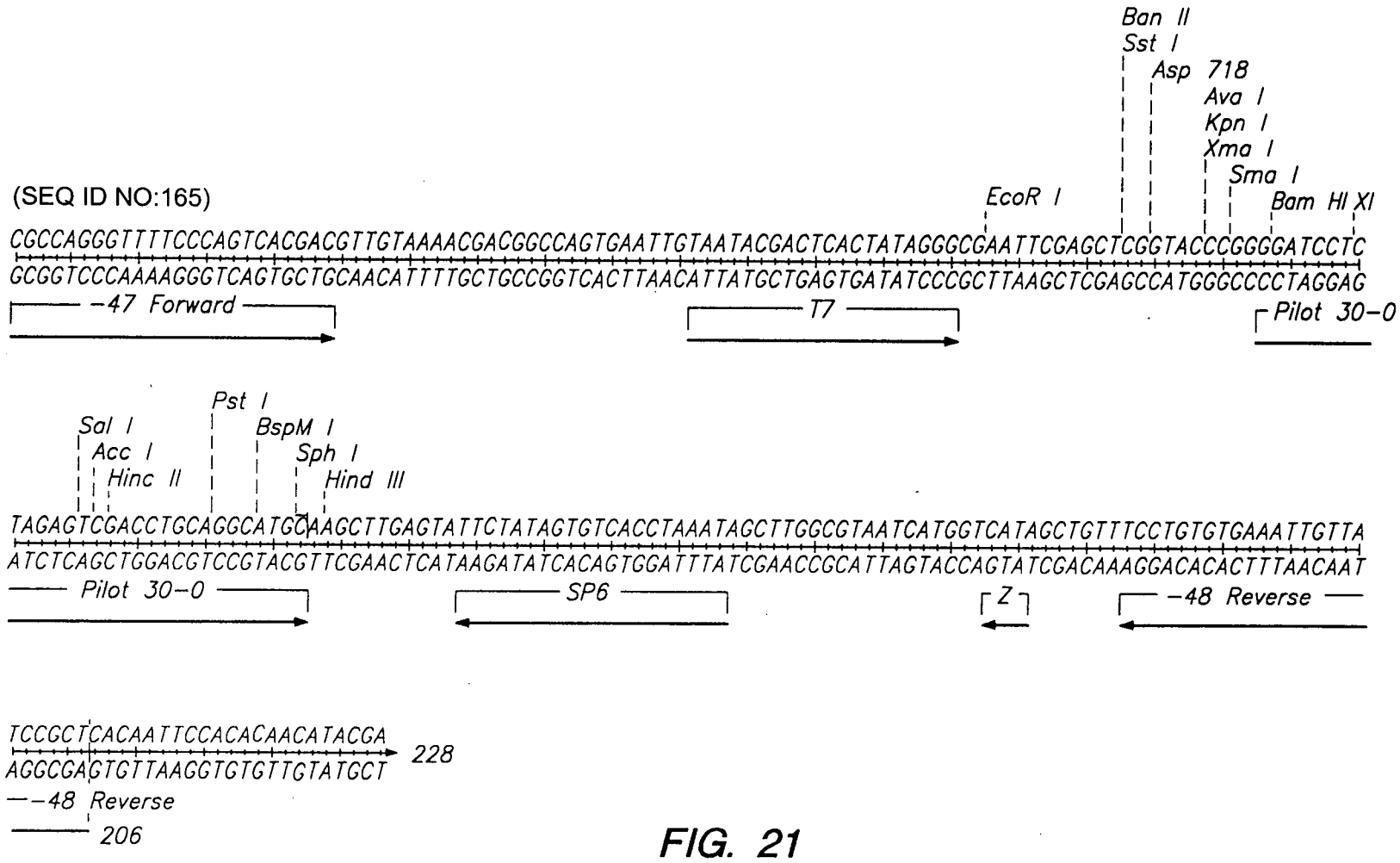

(SEQ ID NO:165)
Ban II
Sst I
Asp 718
Ava I
Kpn I
Xma I
Sma I
Bam HI XI
EcoR I
CGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTC
GCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCACTTAACATTATGCTGAGTGATATCCCGCTTAAGCTCGAGCCATGGGCCCCTAGGAG
-47 Forward
T7
Pilot 30-0
Pst I
Sal I
BspM I
Acc I
Sph I
Hinc II
Hind III
TAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTA
ATCTCAGCTGGACGTCCGTACGTTCGAACTCATAAGATATCACAGTGGATTTATCGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTTAACAAT
Pilot 30-0
SP6
Z
-48 Reverse
TCCGCTCACAATTCCACACAACATACGA
AGGCGAGTGTTAAGGTGTGTTGTATGCT
228
--48 Reverse
206

FIG. 21

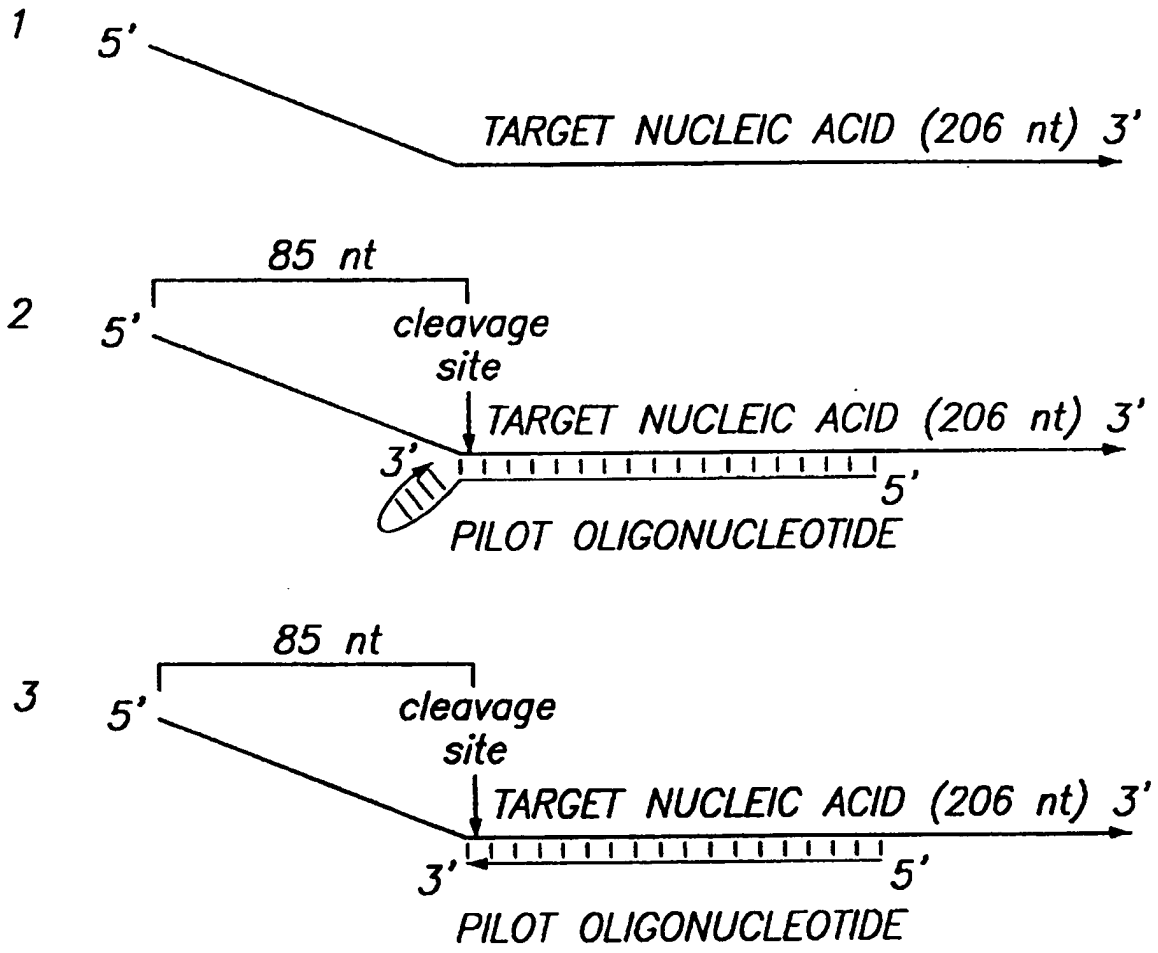

1
5'
TARGET NUCLEIC ACID (206 nt) 3'
85 nt
2
5'
cleavage
site
TARGET NUCLEIC ACID (206 nt) 3'
3'
5'
PILOT OLIGONUCLEOTIDE
85 nt
3
5'
cleavage
site
TARGET NUCLEIC ACID (206 nt) 3'
3'
5'
PILOT OLIGONUCLEOTIDE

FIG. 22A

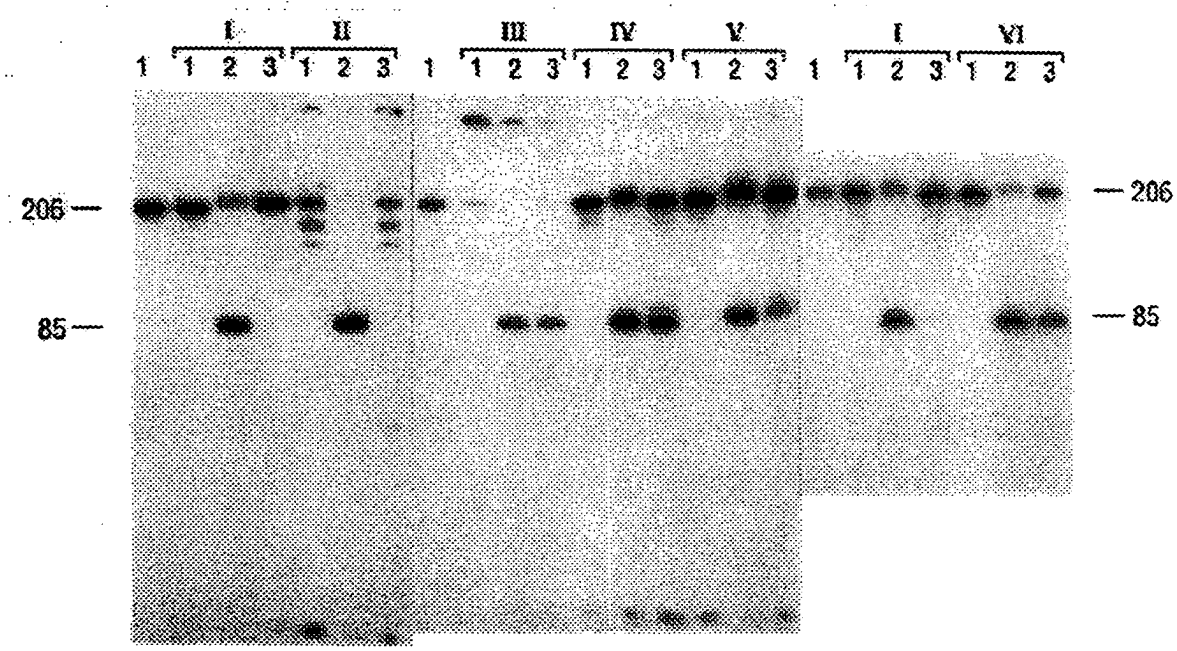



FIG. 22B

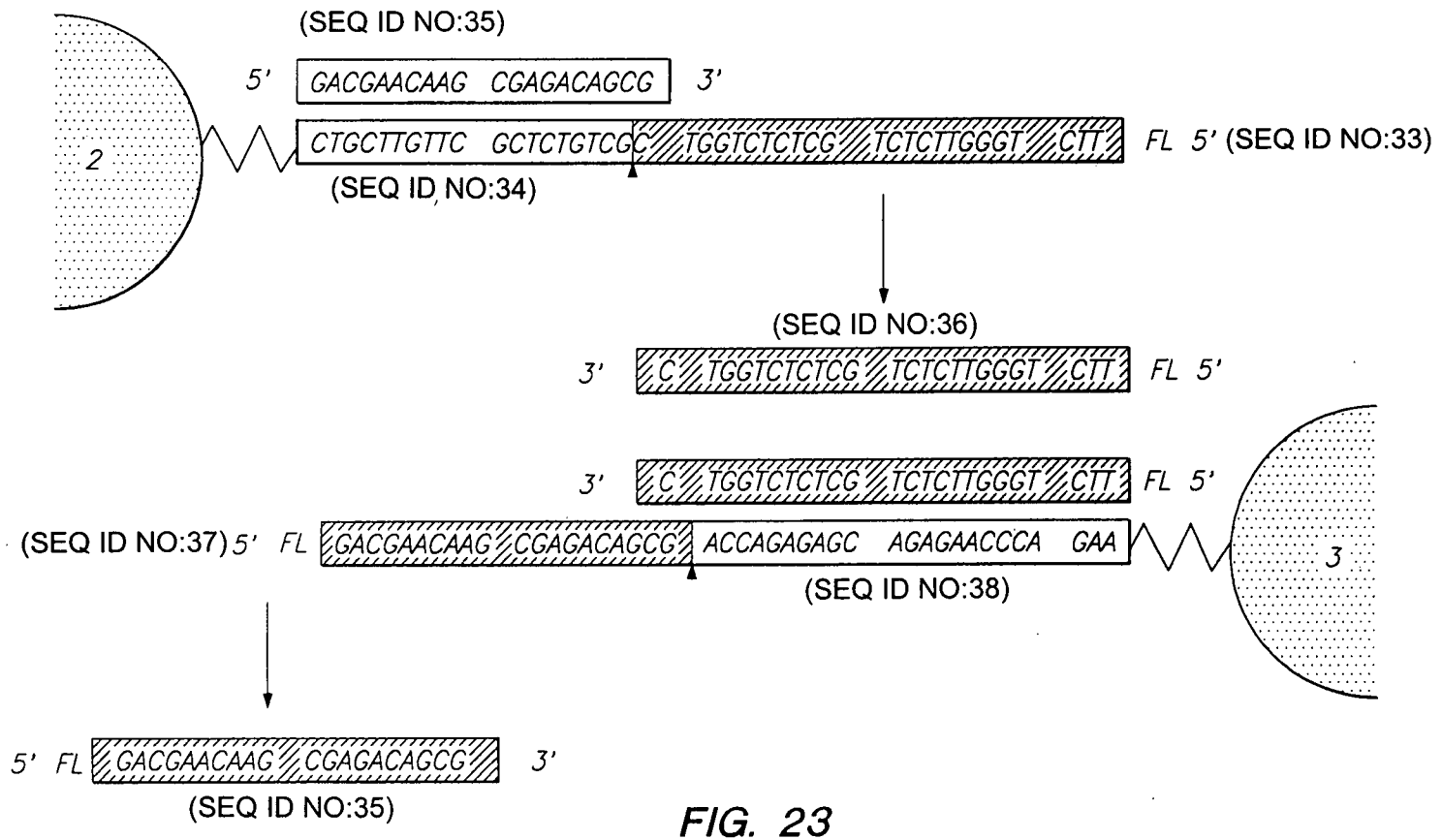

(SEQ ID NO:35)
5'
GACGAACAAG CGAGACAGCG
3'
2
CTGCTTGTTC GCTCTGTCGC
TGGTCTCTCG TCTCTTGGGT CTT
FL 5'
(SEQ ID NO:33)
(SEQ ID NO:34)
(SEQ ID NO:36)
3'
C TGGTCTCTCG TCTCTTGGGT CTT
FL 5'
3'
C TGGTCTCTCG TCTCTTGGGT CTT
FL 5'
(SEQ ID NO:37)
5'
FL
GACGAACAAG CGAGACAGCG
ACCAGAGAGC AGAGAACCCA GAA
3
(SEQ ID NO:38)
5'
FL
GACGAACAAG CGAGACAGCG
3'
(SEQ ID NO:35)

FIG. 23

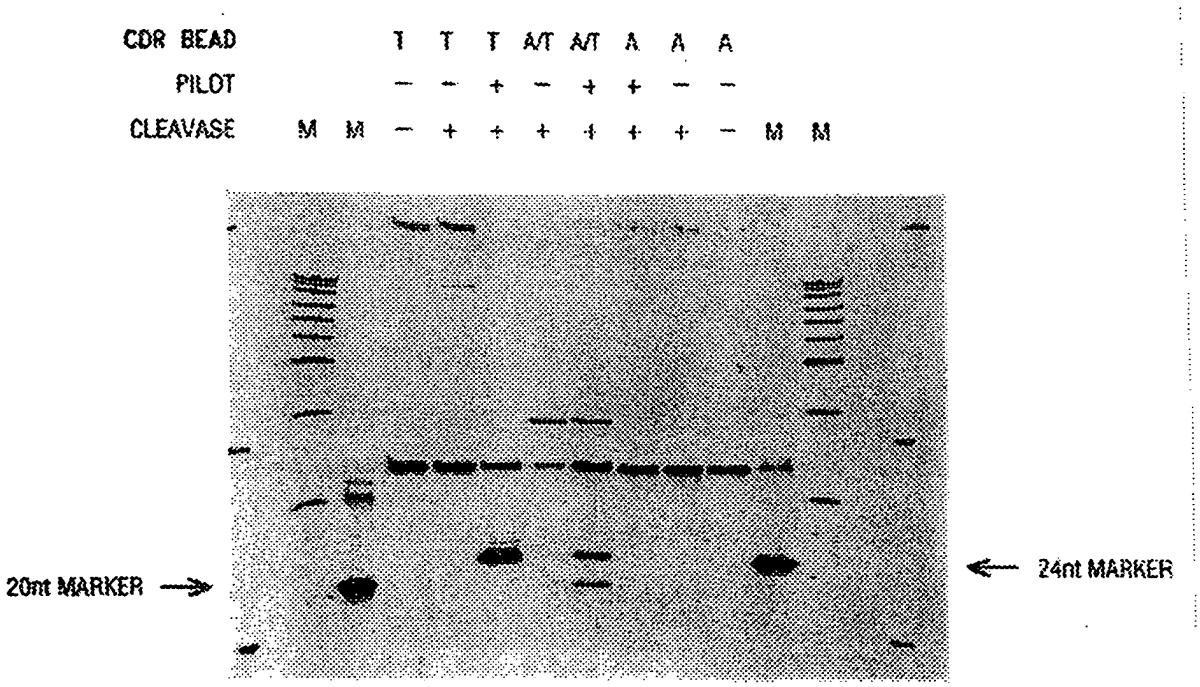

CDR BEAD T T T A/T A/T A A A
PILOT - - + - + + - -
CLEAVASE M M - + + + + + + - M M
20nt MARKER
24nt MARKER

FIG. 24

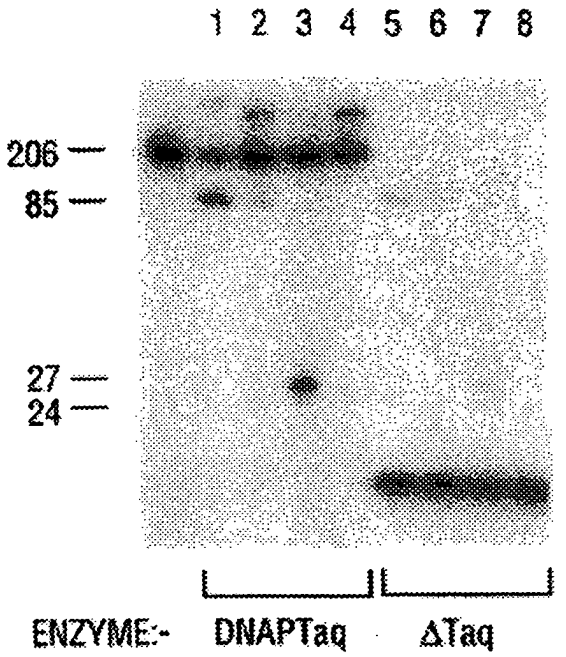



FIG. 25A

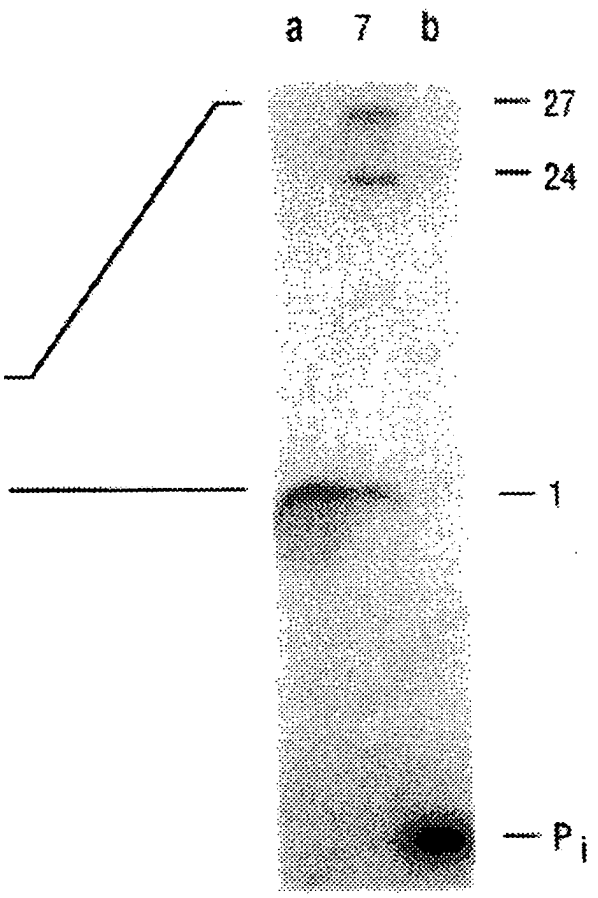



FIG. 25B

FIG. 26A

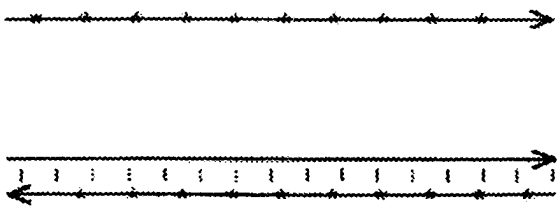
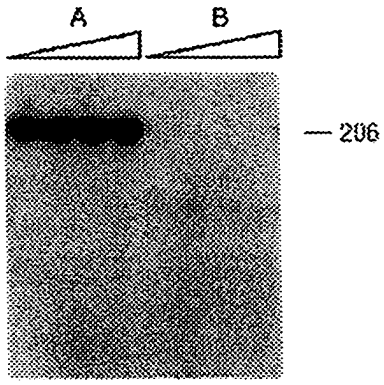


FIG. 26B

* = $^{32}P$



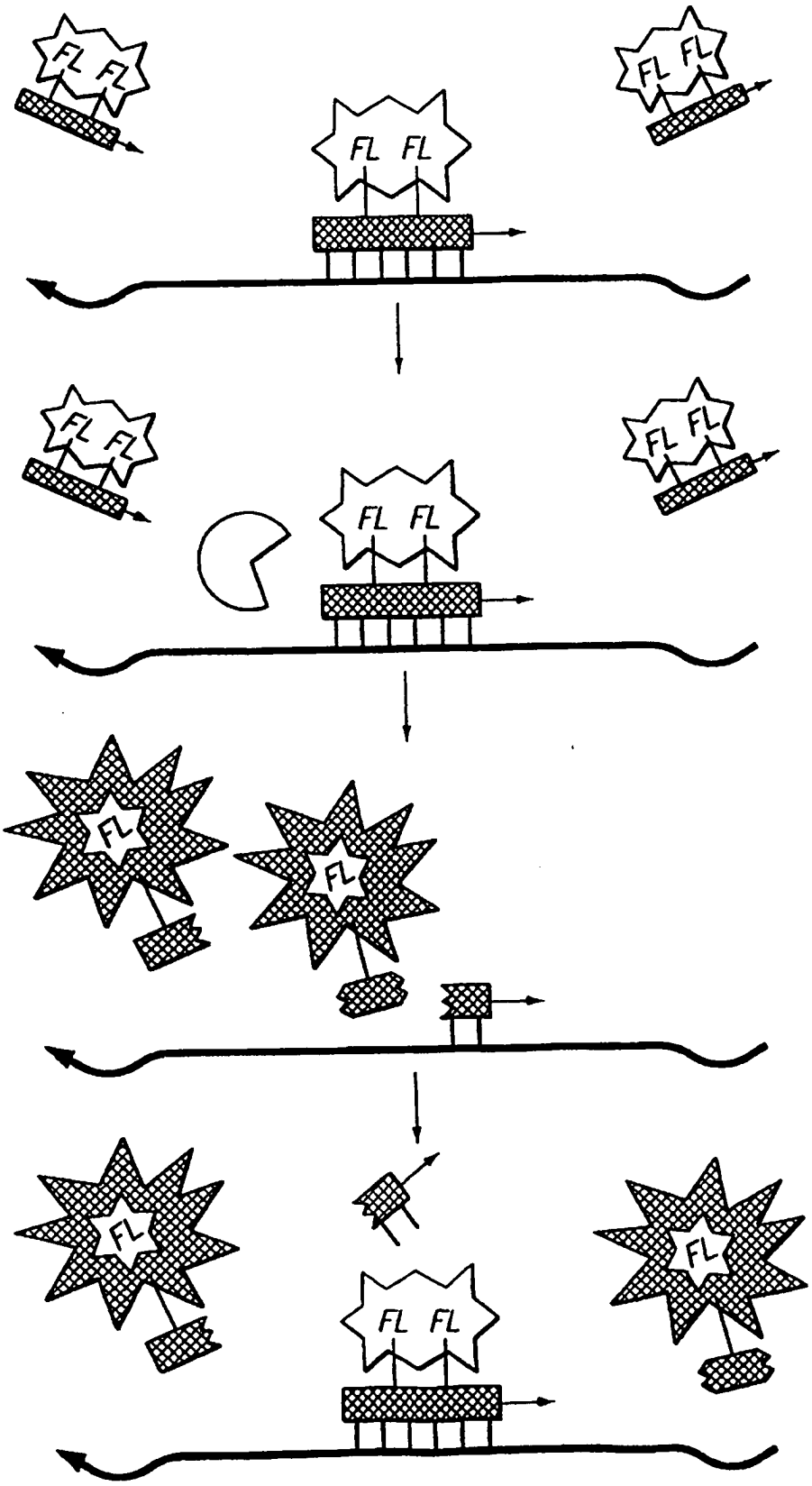



FIG. 27

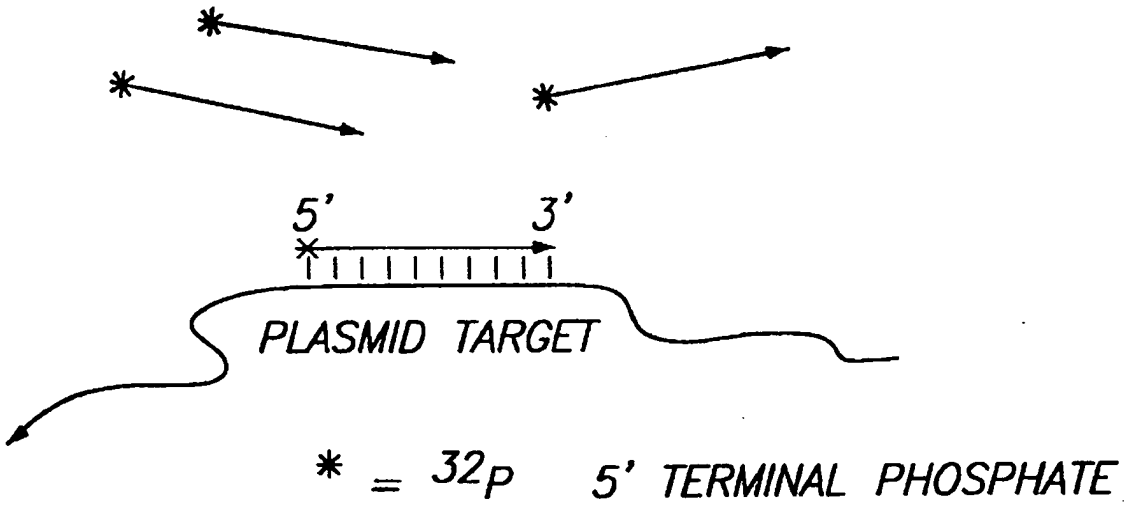



FIG. 28A

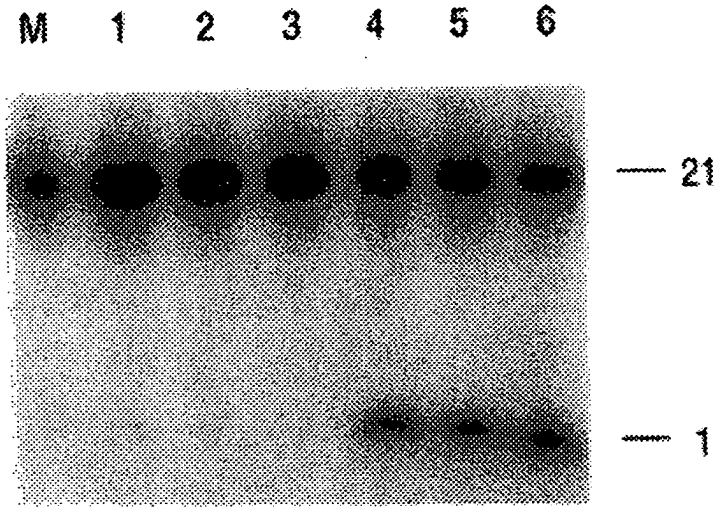



FIG. 28B

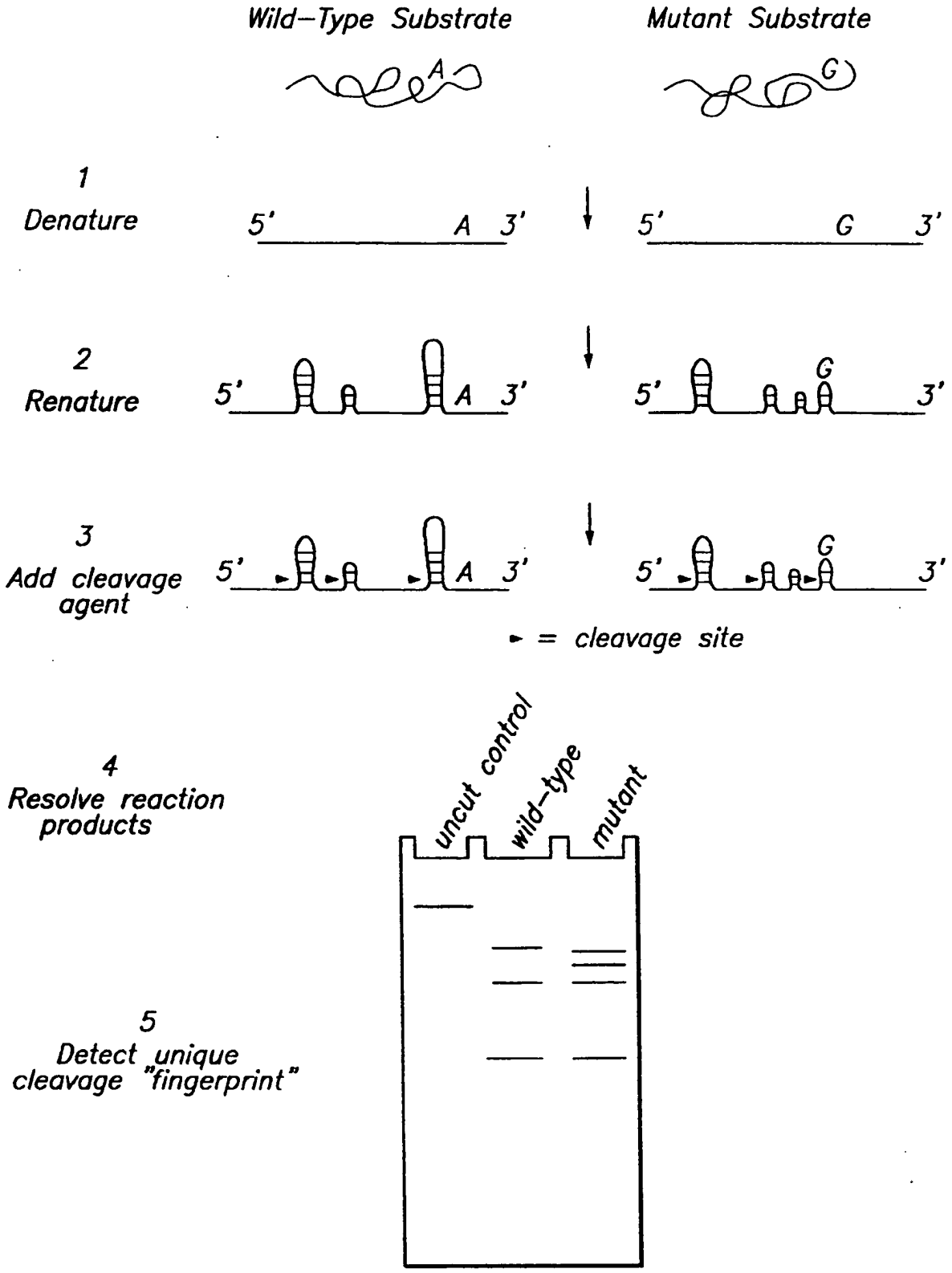

Wild–Type Substrate
Mutant Substrate
A
G
1
Denature
5'
A 3'
5'
G 3'
2
Renature
5'
A 3'
5'
G
3'
3
Add cleavage agent
5'
A 3'
5'
G
3'
► = cleavage site
4
Resolve reaction products
uncut control
wild–type
mutant
5
Detect unique cleavage "fingerprint"

FIG. 29

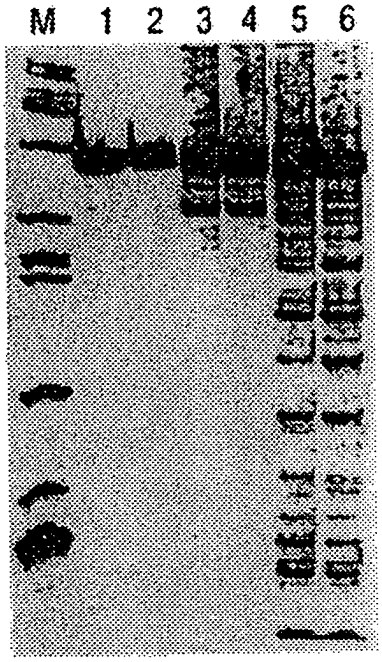



FIG. 30

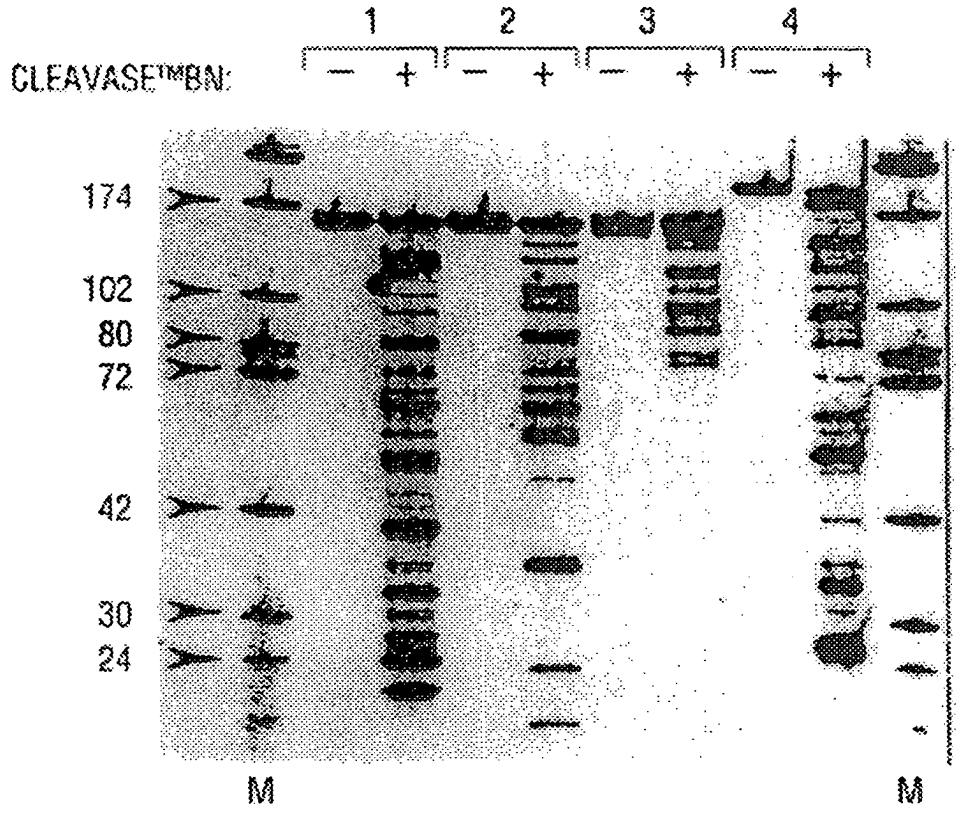



FIG. 31

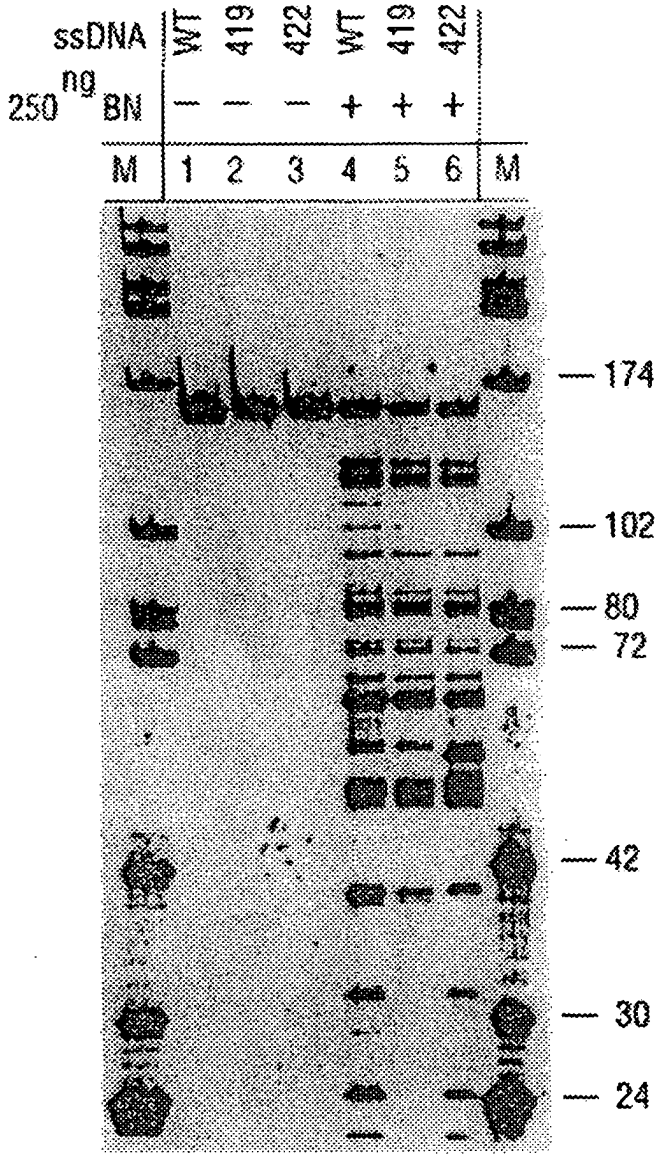

ssDNA
WT
419
422
WT
419
422
250 ng BN
— — — + + +
M 1 2 3 4 5 6 M
174
102
80
72
42
30
24

FIG. 32

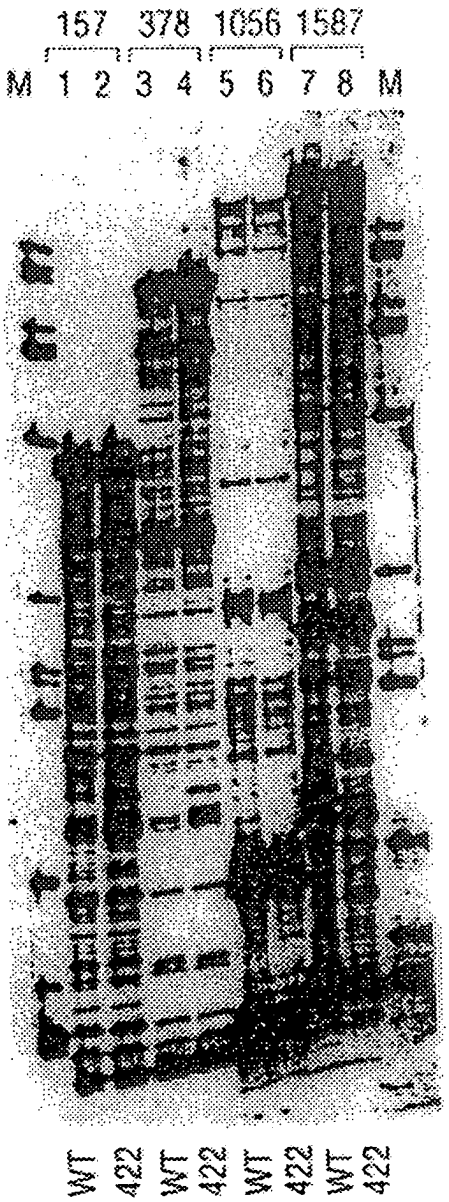



FIG. 33

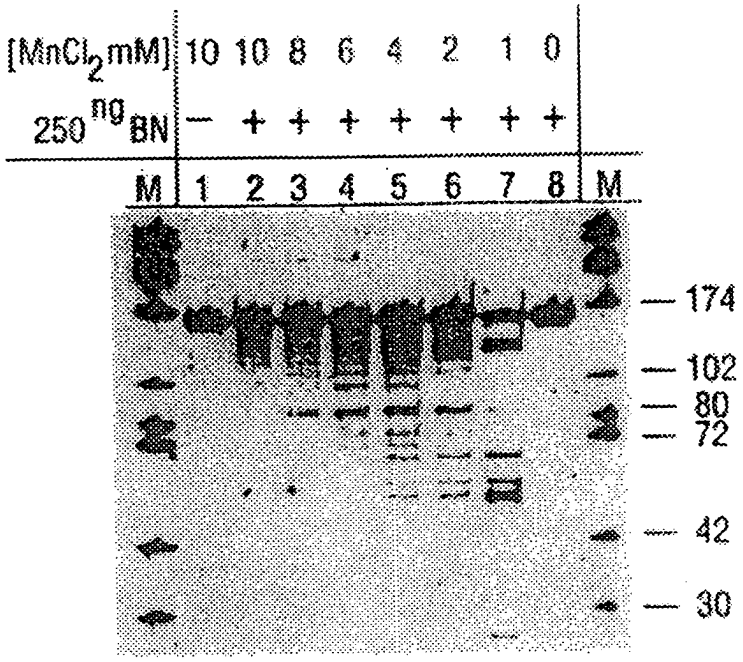



FIG. 34

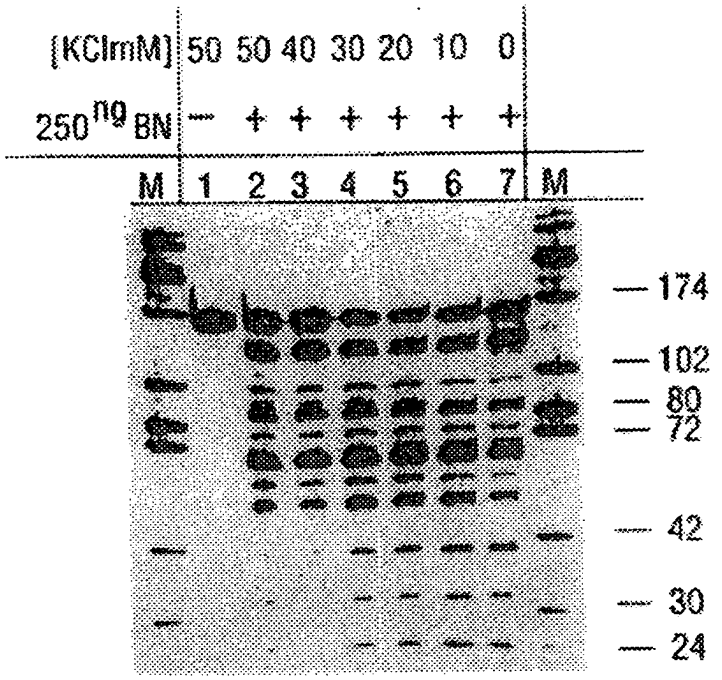



FIG. 35

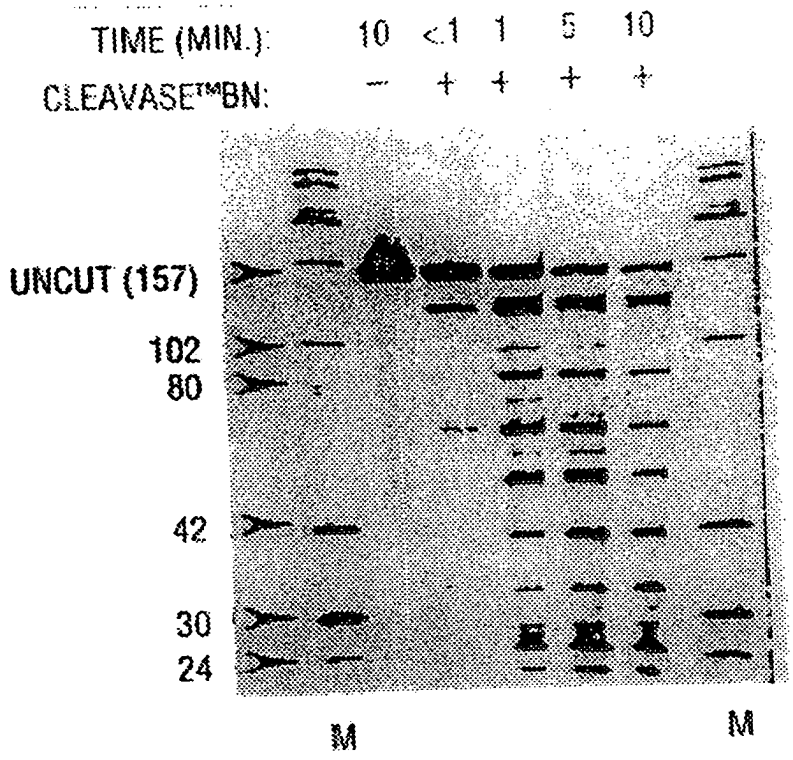



FIG. 36

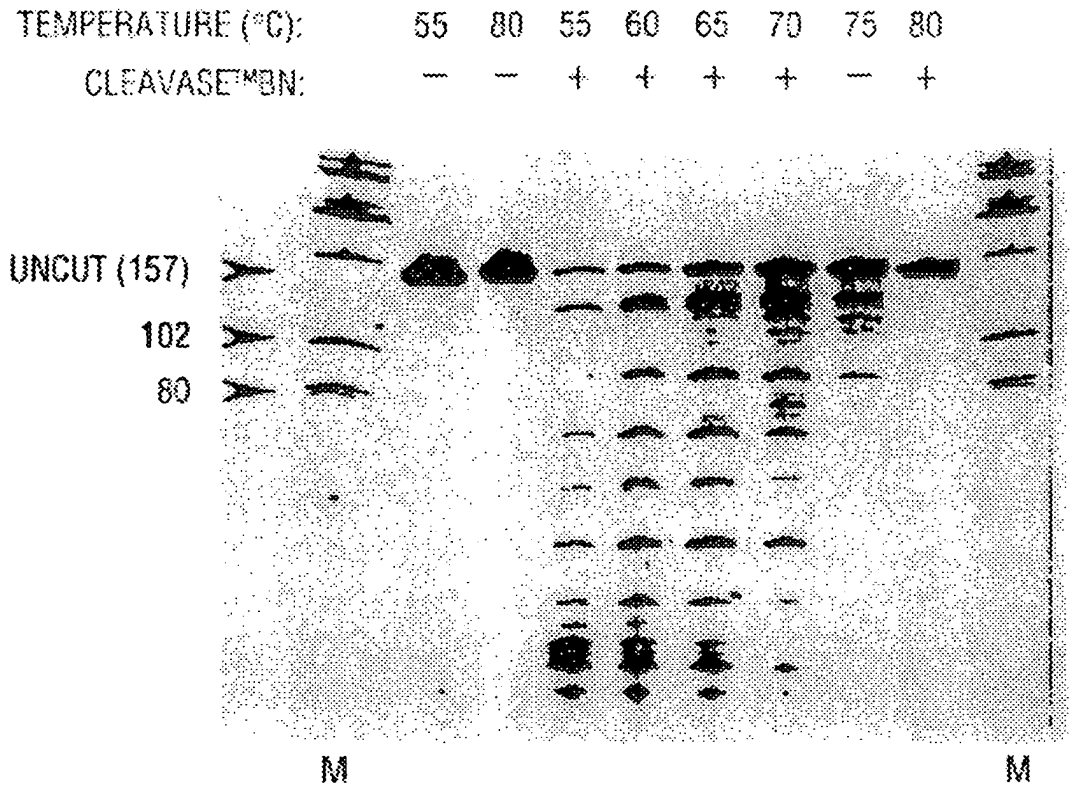

TEMPERATURE (°C): 55 80 55 60 65 70 75 80
CLEAVASE™BN: — — + + + + — +
UNCUT (157)
102
80
M
M

FIG. 37

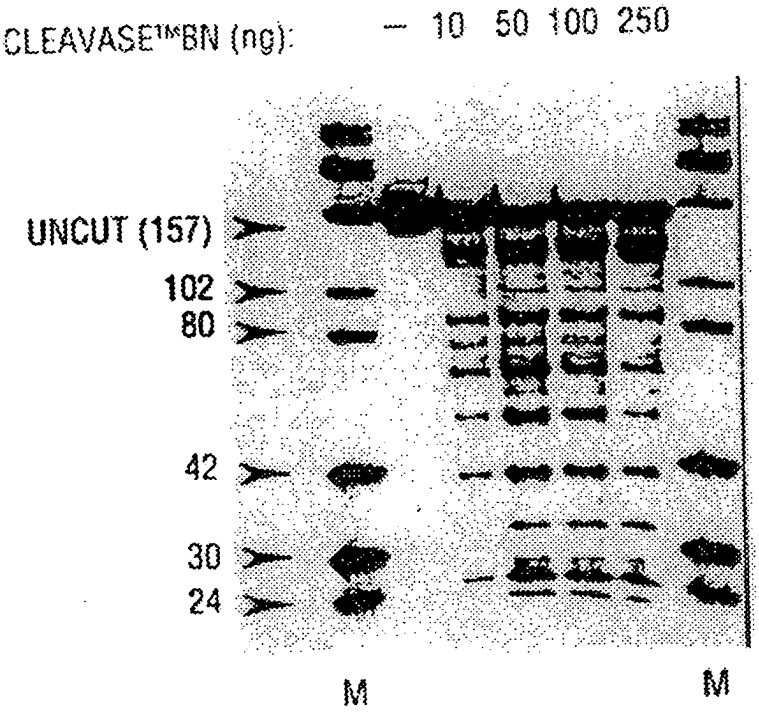



FIG. 38

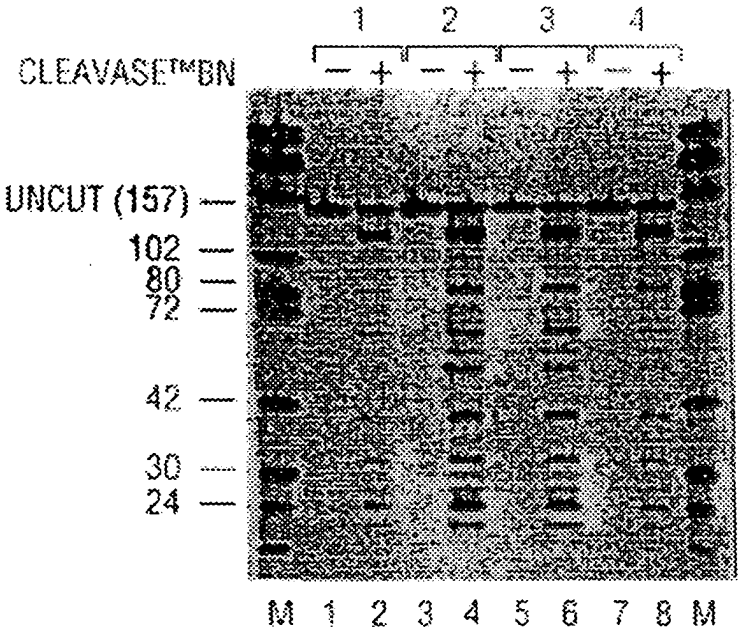



FIG. 39

| STRAND | 5' - BIOTIN SENSE STRAND | | | | | | 5' - FLUORESCEIN ANTI-SENSE STRAND | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ssDNA | WT | 419 | 422 | WT | 419 | 422 | WT | 419 | 422 | WT | 419 | 422 |
| 250 ng BN | – | – | – | + | + | + | + | + | + | – | – | – |
| M | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

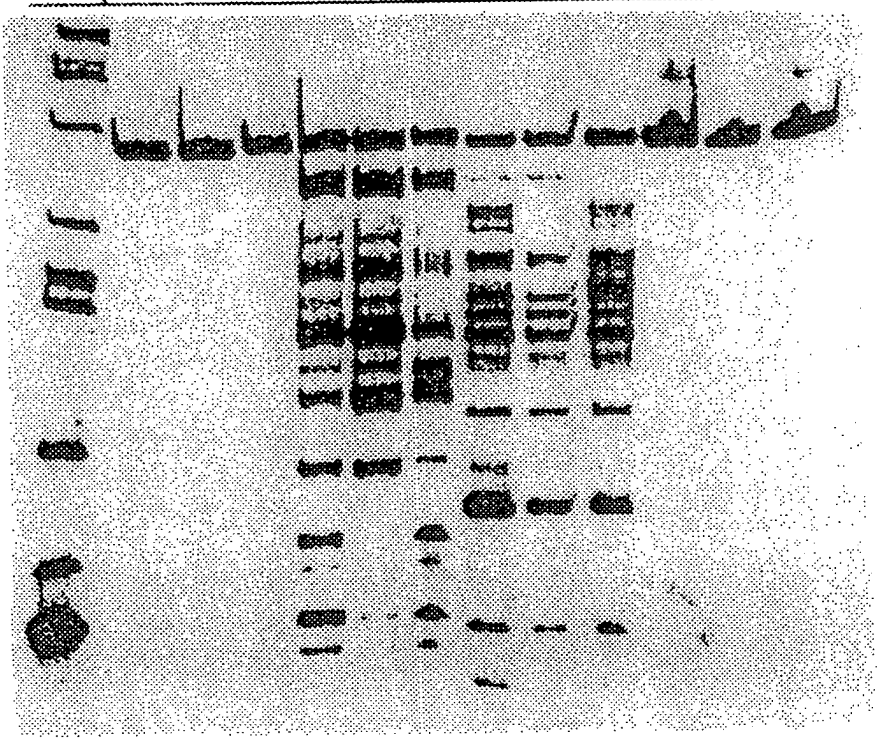


FIG. 40

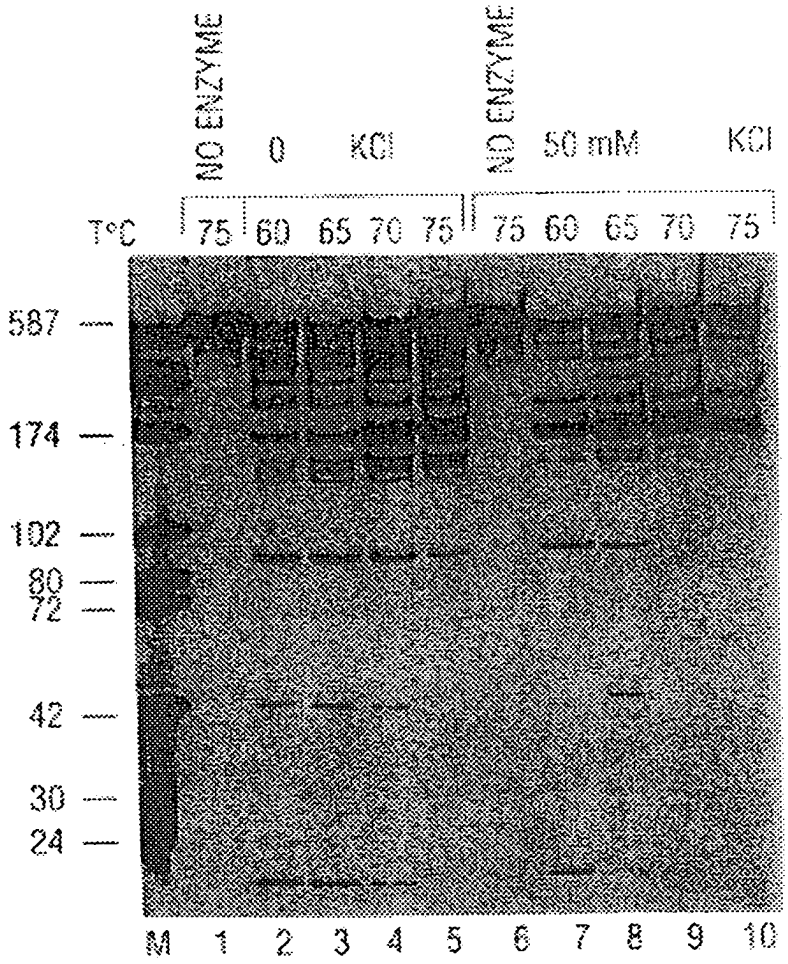

NO ENZYME
0
KCl
NO ENZYME
50 mM
KCl
T°C
75
60
65
70
75
75
60
65
70
75
587
174
102
80
72
42
30
24
M
1
2
3
4
5
6
7
8
9
10

FIG. 41

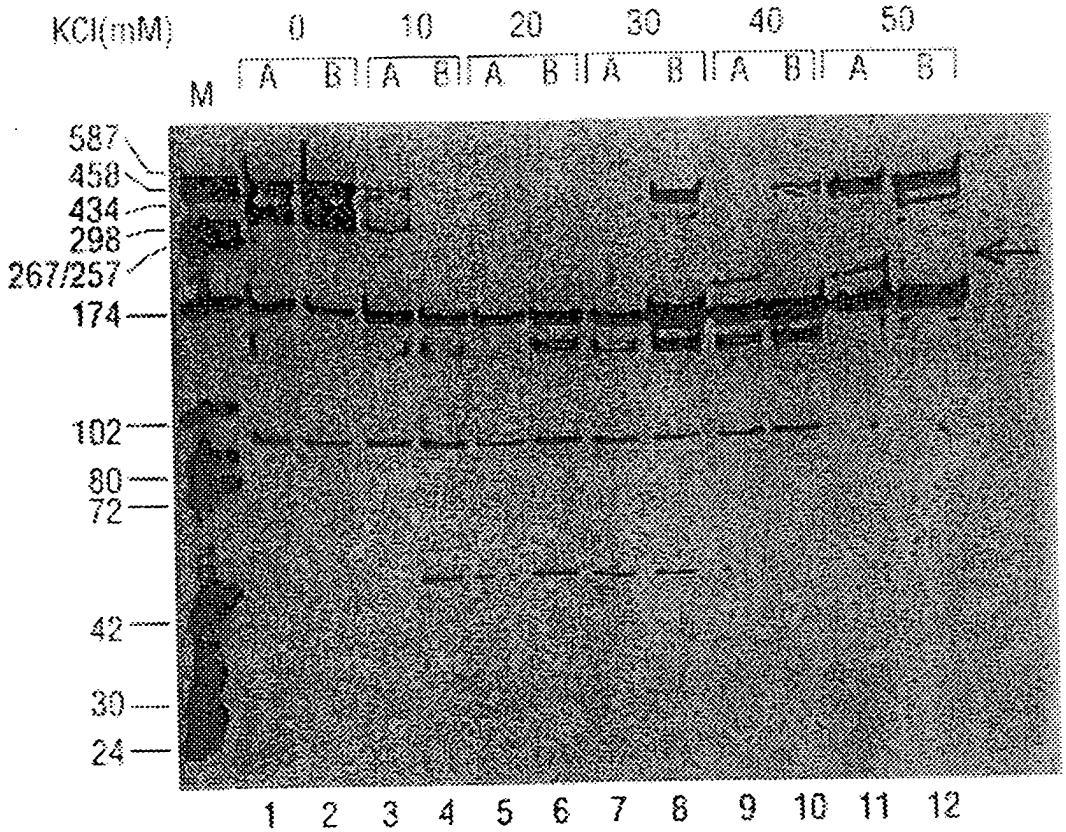

KCl(mM)
0
10
20
30
40
50
A
B
M
587
458
434
298
267/257
174
102
80
72
42
30
24
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 42

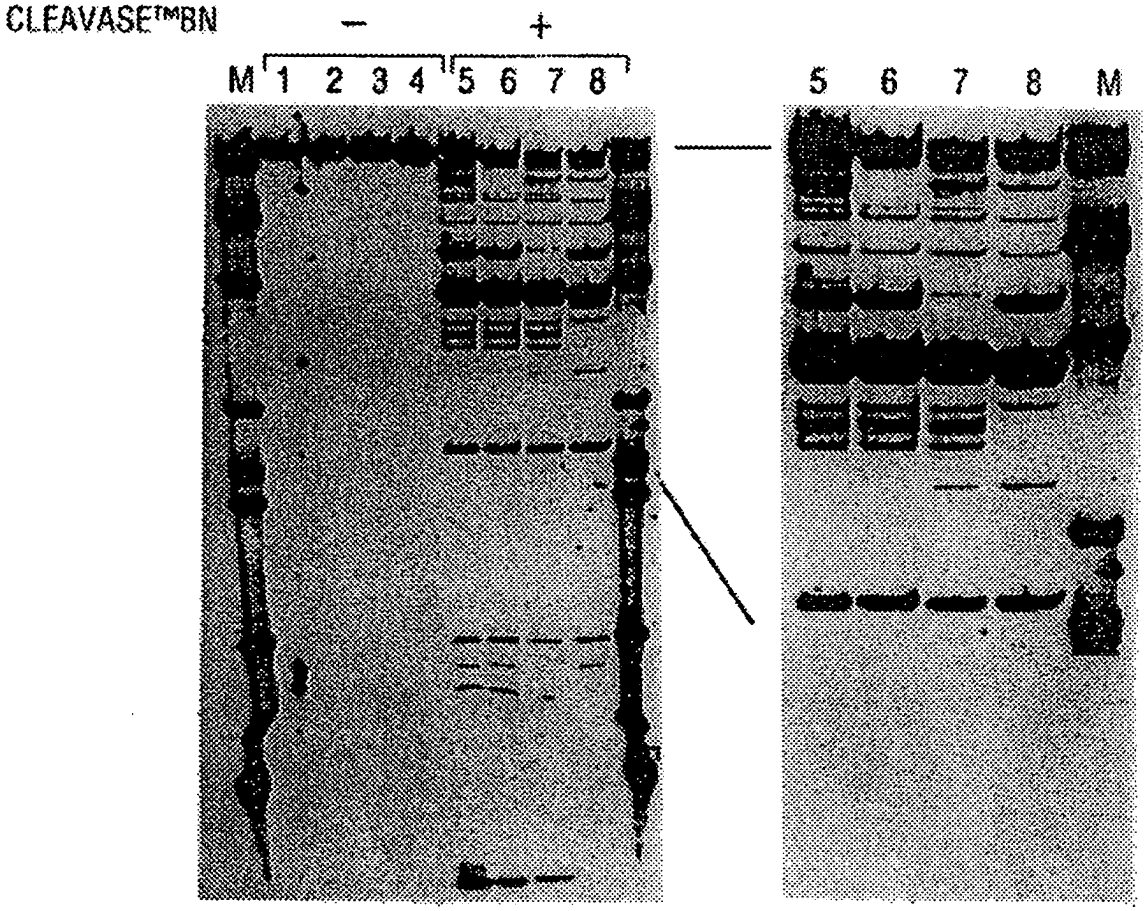



FIG. 43

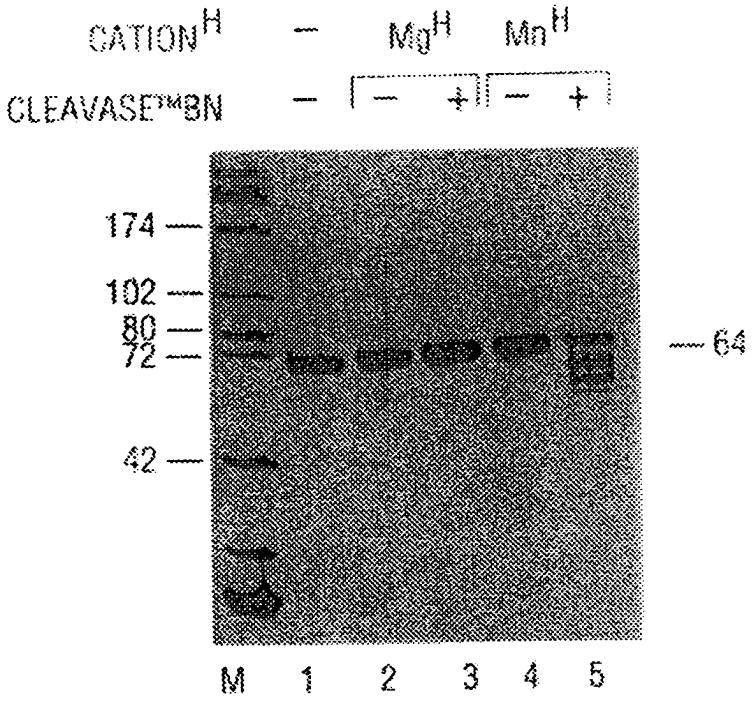



FIG. 44

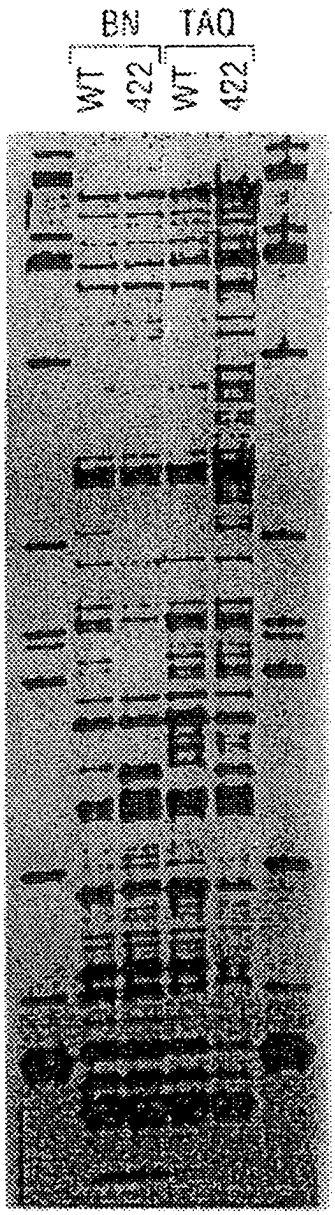



FIG. 45

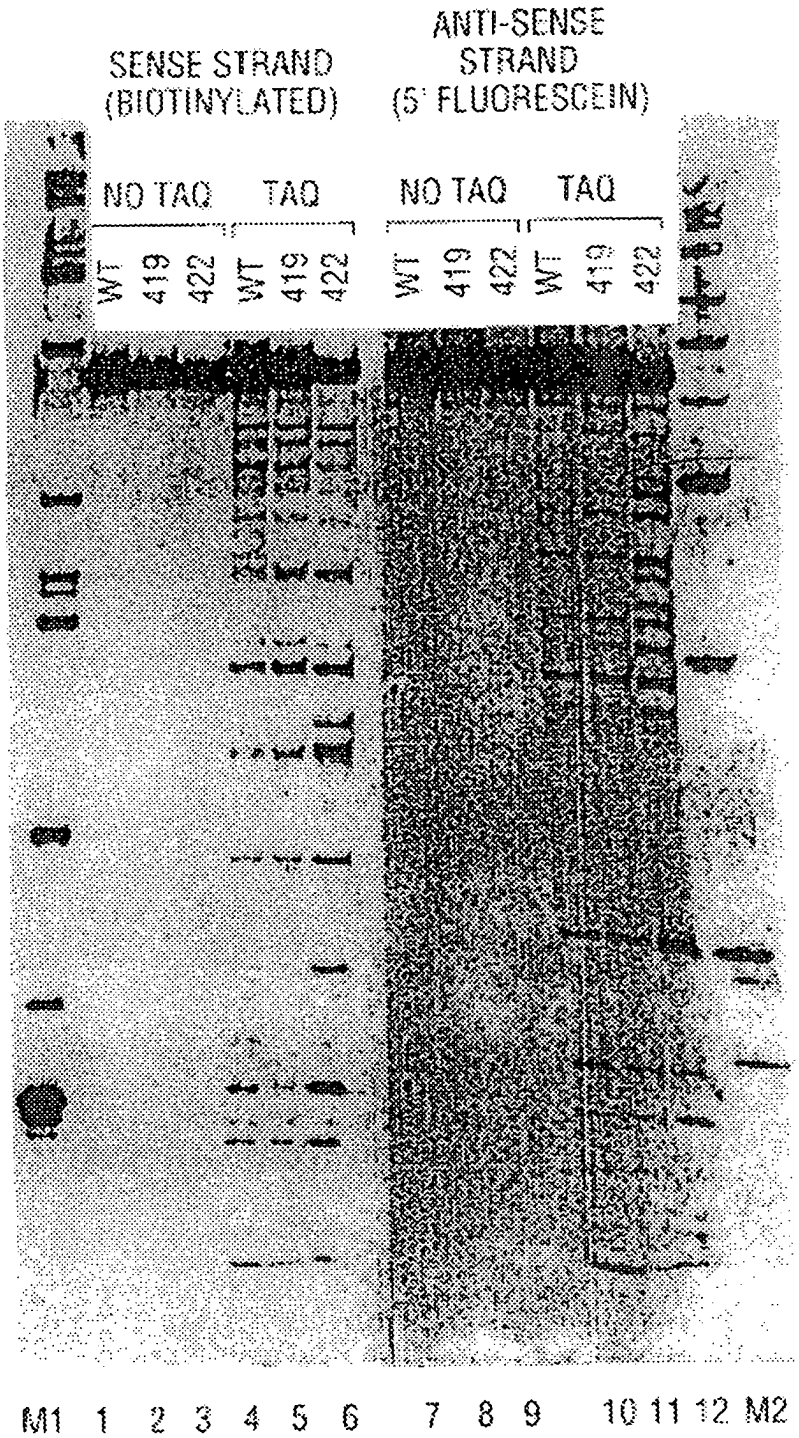

SENSE STRAND
(BIOTINYLATED)
ANTI-SENSE
STRAND
(5' FLUORESCEIN)
NO TAQ
TAQ
NO TAQ
TAQ
WT
419
422
WT
419
422
WT
419
422
WT
419
422
M1 1 2 3 4 5 6 7 8 9 10 11 12 M2

FIG. 46

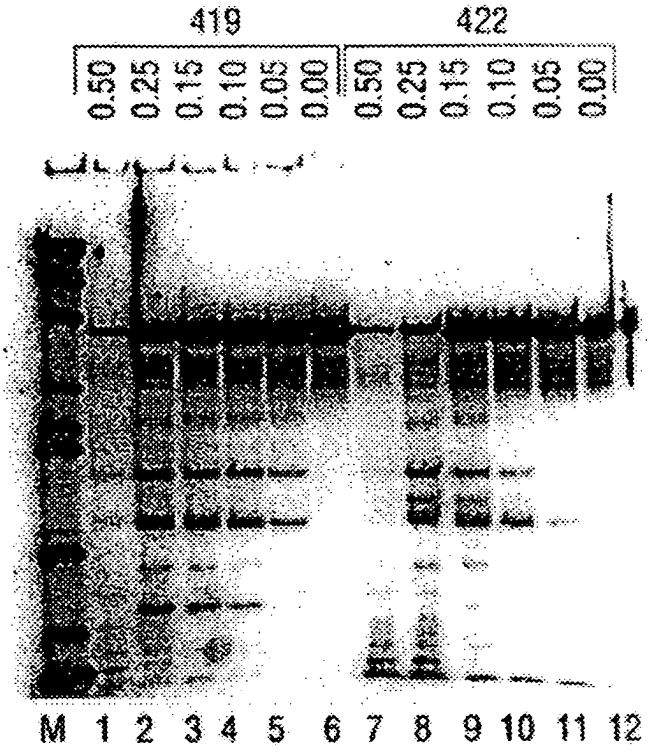

419
422
0.50
0.25
0.15
0.10
0.05
0.00
0.50
0.25
0.15
0.10
0.05
0.00
M 1 2 3 4 5 6 7 8 9 10 11 12

FIG. 47

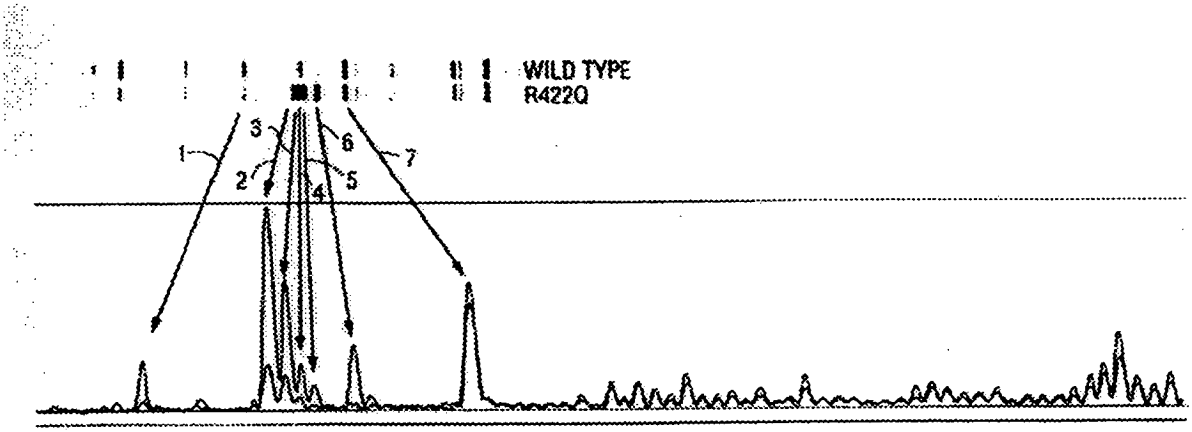



FIG. 48

| | |
|---|---|
| L.100.8-1 | 5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG |
| (SEQ ID NO: 76) | 3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC |
| L.46.16-10 | 5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGACTTTCCACAAGGGG |
| (SEQ ID NO: 77) | 3'CCGACTGTTCTTCCTTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC |
| L.46.16-12 | 5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGACTTTCCACAAGGGG |
| (SEQ ID NO: 78) | 3'CCGACTGTTCTTCCTTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC |
| L19.16-3 | 5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG |
| (SEQ ID NO: 79) | 3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC |
| L.CEM/251 | 5'GGCTGACAAGAAGGAAACTCGCTGAAACAGCAGGGACTTTCCACAAGGGG |
| (SEQ ID NO: 80) | 3'CCGACTGTTCTTCCTTTGAGCGACTTTGTCGTCCCTGAAAGGTGTTCCCC |
| L.36.8-3 | 5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG |
| (SEQ ID NO: 81) | 3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC |

FIG. 49A

```
                                                                  100
L.100.8-1          ATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO: 76)    TACAATGCCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAGAGA

L.46.16-10         ATGTTATGGGGAGG----------AGCCGGTCGGGAACACCCACTTTCT
(SEQ ID NO: 77)    TACAATACCCCTCC----------TCGGCCAGCCCTTGTGGGTGAAAGA

L.46.16-12         ATGTTATGGGGAGG----------AGCCGGTCGGGAACACCCACTTTCT
(SEQ ID NO: 78)    TACAATACCCCTCC----------TCGGCCAGCCCTTGTGGGTGAAAGA

L19.16-3           ATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCCCTCTCT
(SEQ ID NO: 19)    TACAATGCCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGGGAGAGA

L.CEM/251          ATGTTACGGGGAGGTACTGGGAAGGAGCCGGTCGGGAACGCCCACTTTCT
(SEQ ID NO: 80)    TACAATGCCCCTCCATGACCCTTCCTCGGCCAGCCCTTGCGGGTGAAAGA

L.36.8-3           ATGTTACGGAGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO: 81)    TACAATGCCTCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAGAGA
```

FIG. 49B

150

| | |
|---|---|
| L.100.8-1 | 5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA |
| | 3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT |
| L.46.16-10 | 5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA |
| | 3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT |
| L.46.16-12 | 5'TGGTGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA |
| | 3'ACCACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT |
| L.19.16-3 | 5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA |
| | 3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT |
| L.CEM/251 | 5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA |
| | 3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT |
| L.36.8-3 | 5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA |
| | 3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT |

FIG. 49C

200

| | |
|---|---|
| L.100.8-1 | GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG |
| | CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC |
| L.46.16-10 | GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGCTAG |
| | CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC |
| L.46.16-12 | GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG |
| | CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC |
| L.19.16-3 | GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG |
| | CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC |
| L.CEM/251 | GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG |
| | CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC |
| L.36.8-3 | GAGGCTGGCAGATTGAGCCCTAGGAGGTTCTCTCCAGCACTAGCAGGTAG |
| | CTCCGACCGTCTAACTCGGGATCCTCCAAGAGAGGTCGTGATCGTCCATC |

**FIG. 49D**

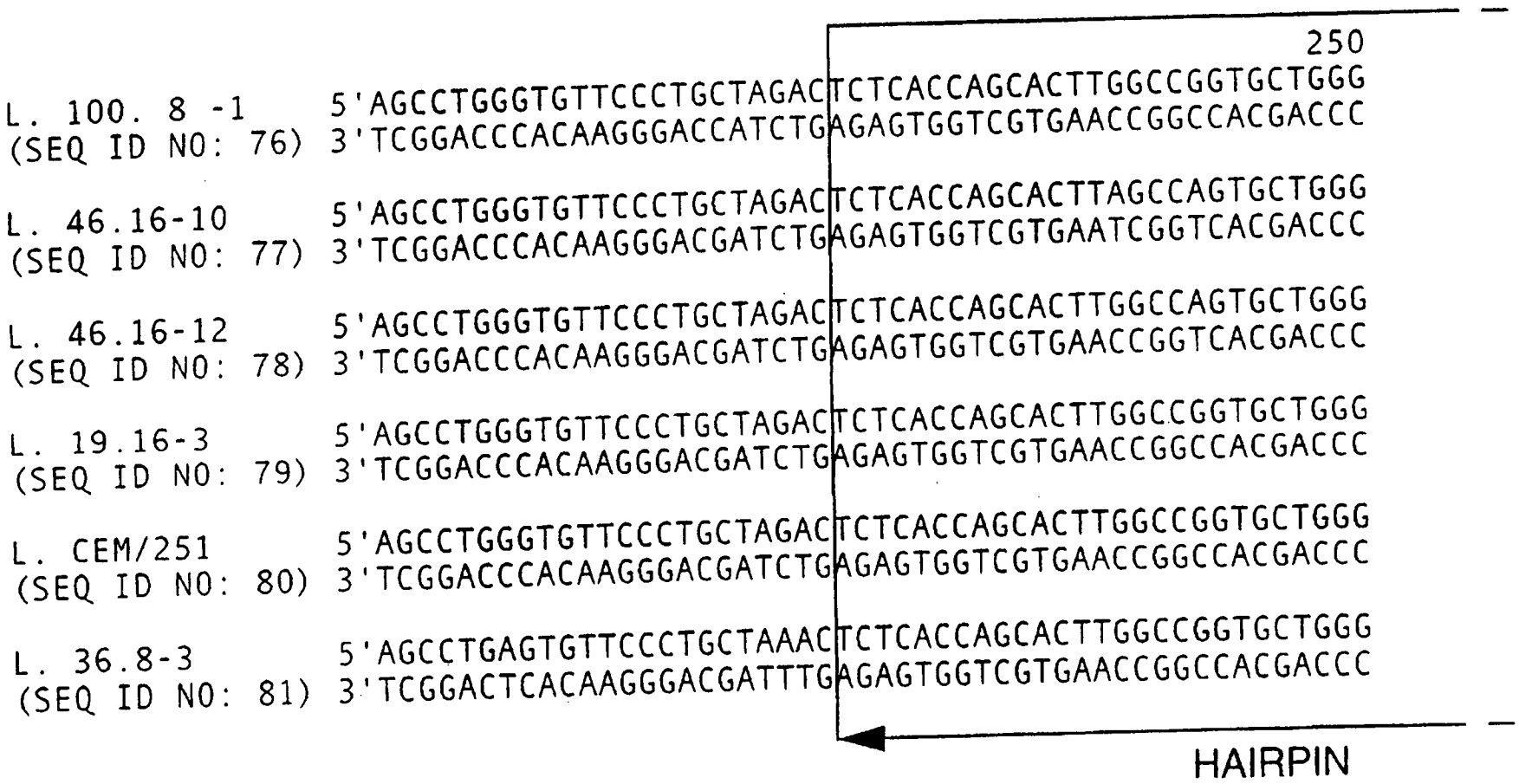



FIG. 49E

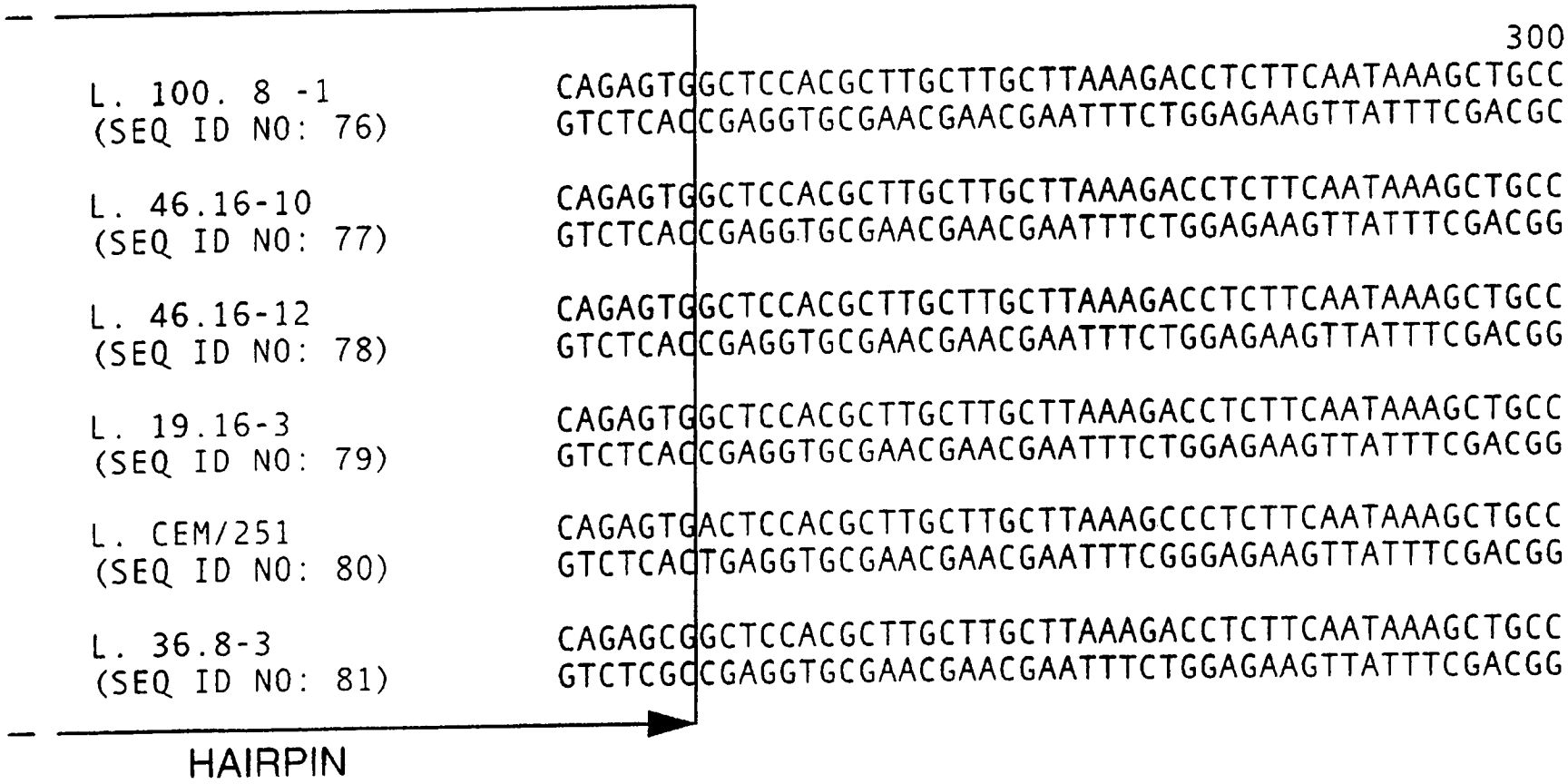



FIG. 49F

```
                                                                  350
L.100.8-1       5'ATTTTAGAAGTAGGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG  G 3'
                3'TAAAATCTTCATCCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC  C 5'

L.46,16-10      5'ATTTTAGAAGTAAGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG  G 3'
                3'TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC  C 5'

L.46.16-12      5'ATTTTAGAAGTAAGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG  G 3'
                3'TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC  C 5'

L.19,16-3       5'ATTTTAGAAGTAGGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG  G 3'
                3'TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC  C 5'

L.CEM/251       5'ATTTTAGAAGTAAGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG  G 3'
                3'TAAAATCTTCATTCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC  C 5'

L.36.8-3        5'ATTTTAGAAGTAGGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG  G 3'
                3'TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC  C 5'
```

FIG. 49G


FIG. 50

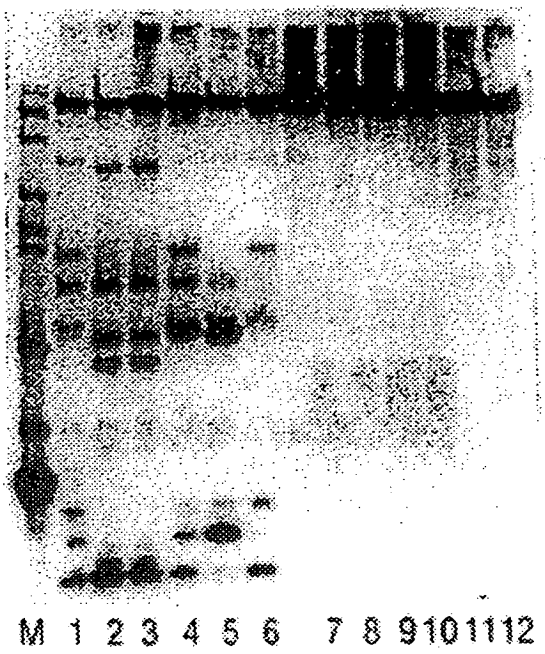



FIG. 51

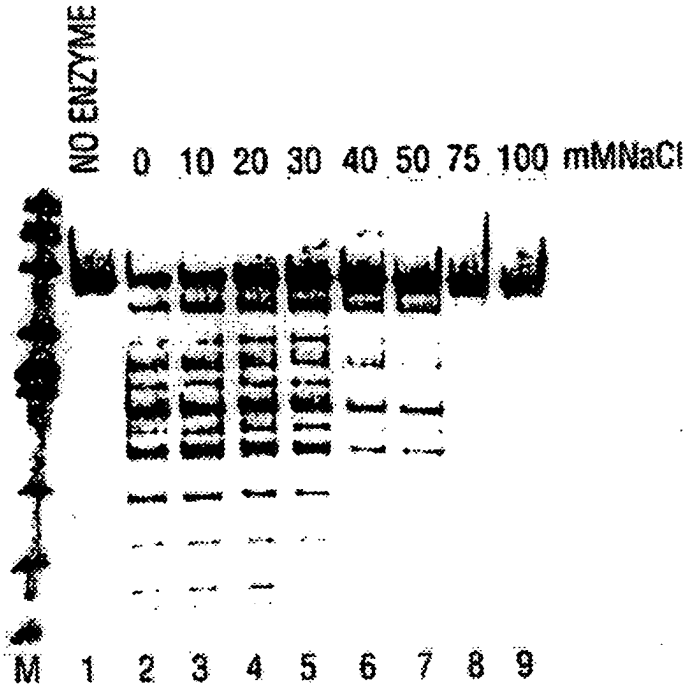



FIG. 52

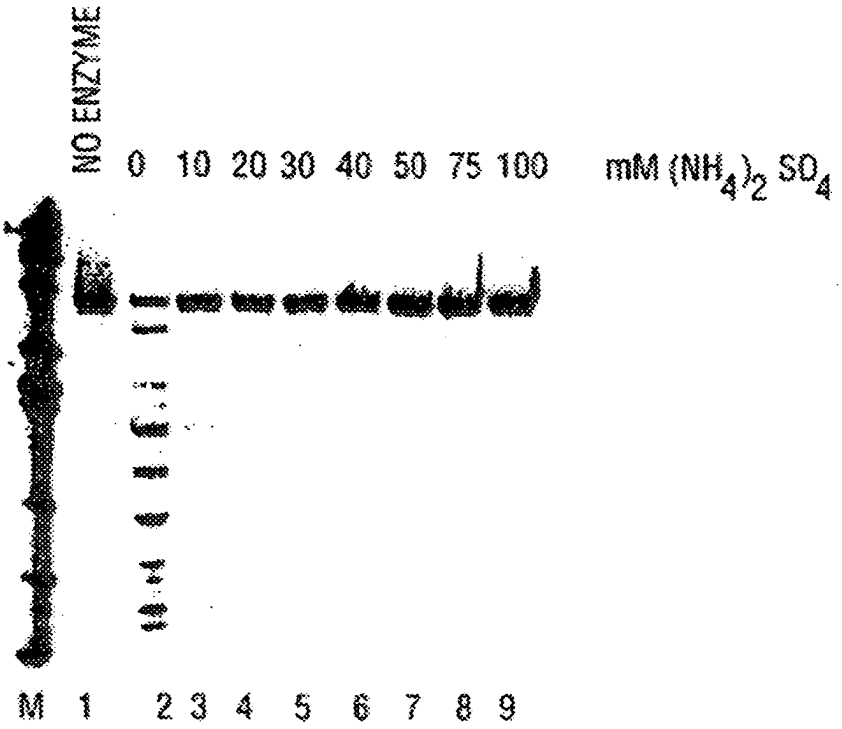



FIG. 53

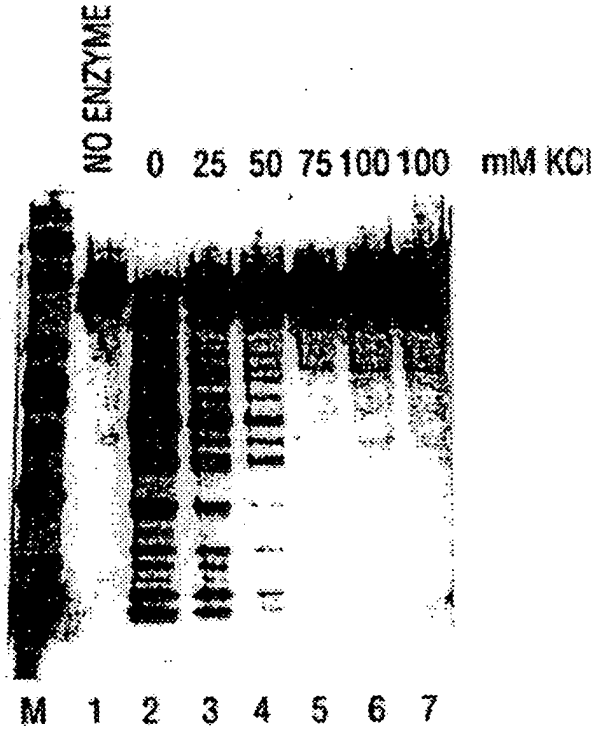



FIG. 54

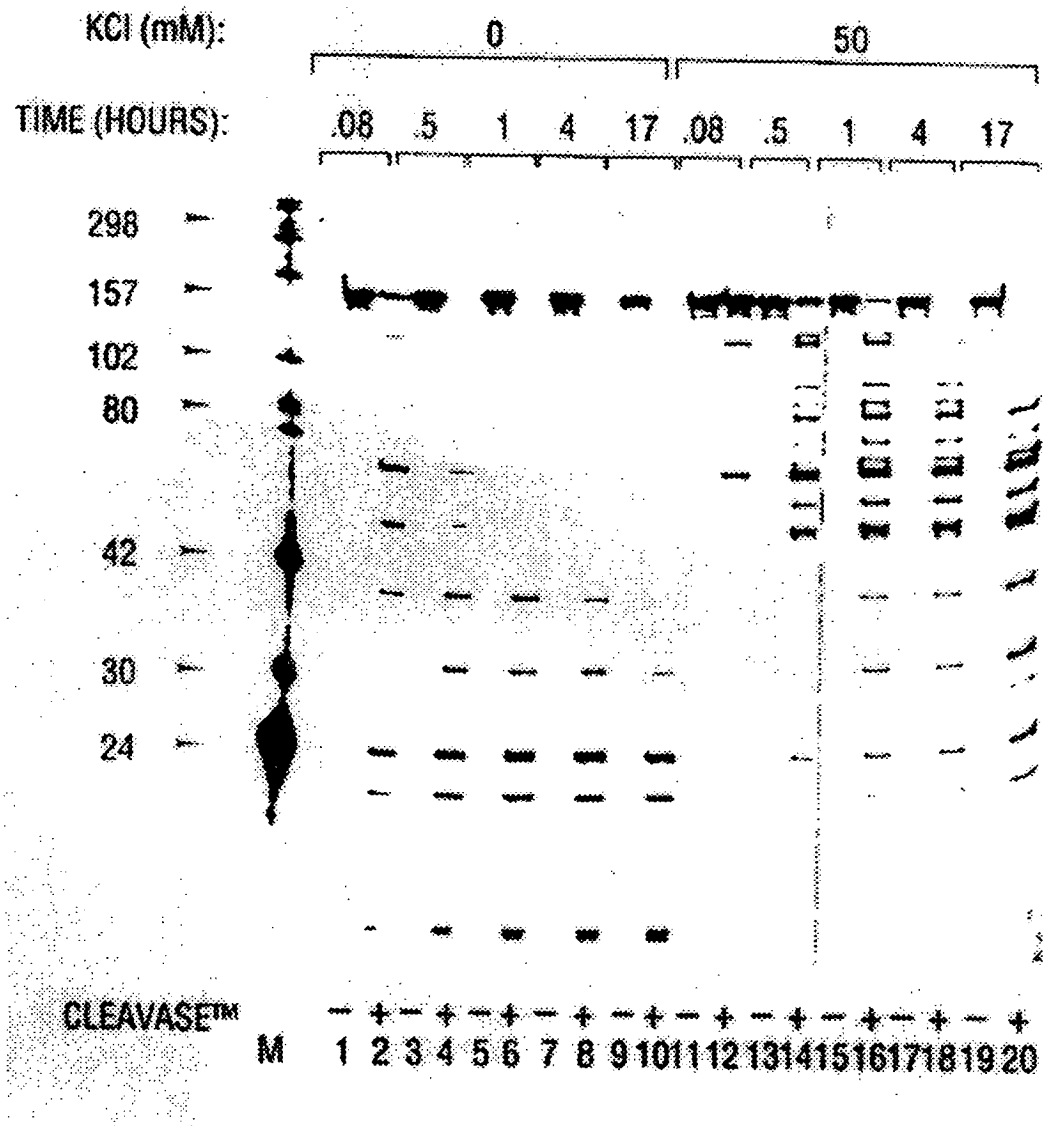

KCl (mM):
0
50
TIME (HOURS):
.08 .5 1 4 17 .08 .5 1 4 17
298
157
102
80
42
30
24
CLEAVASE™
M
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 55

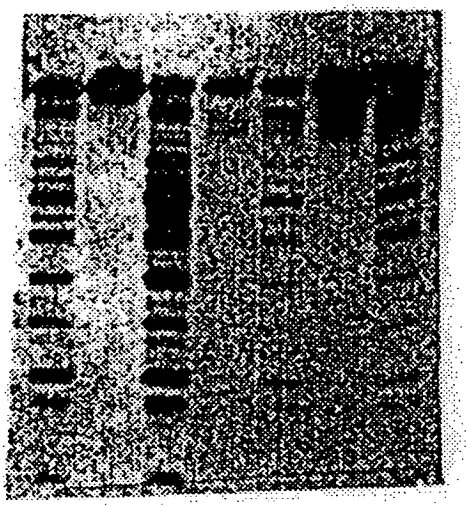



FIG. 56

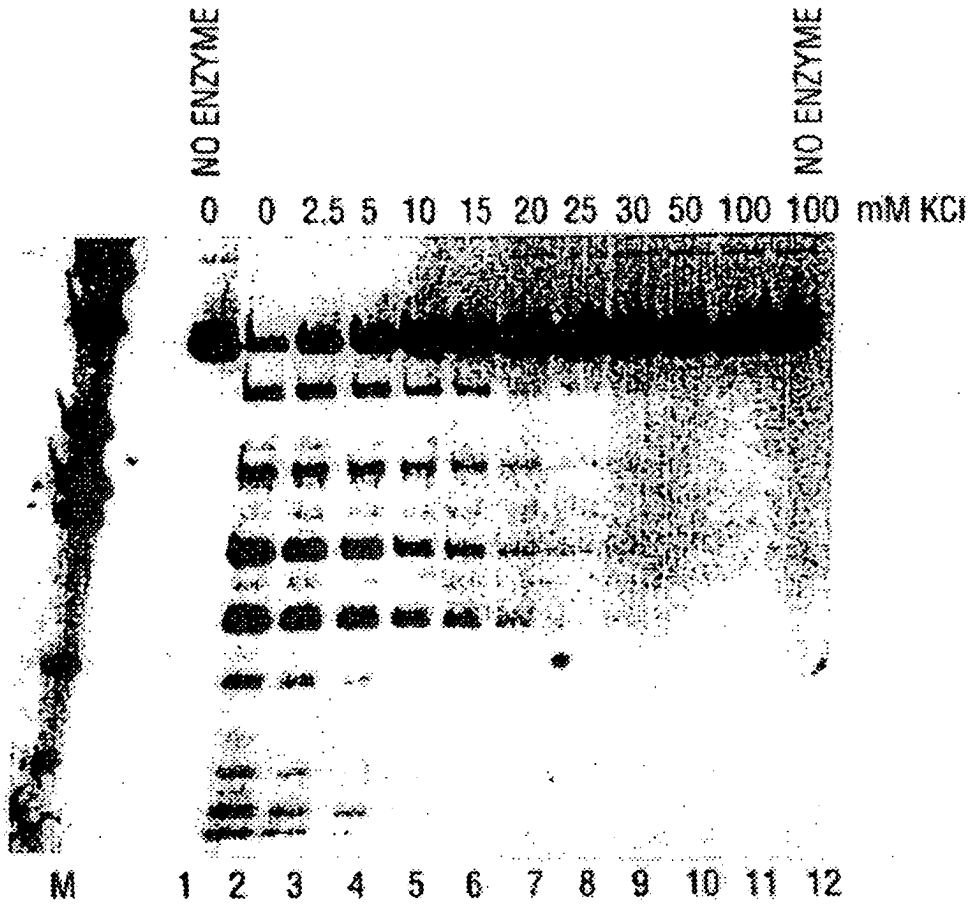



FIG. 57

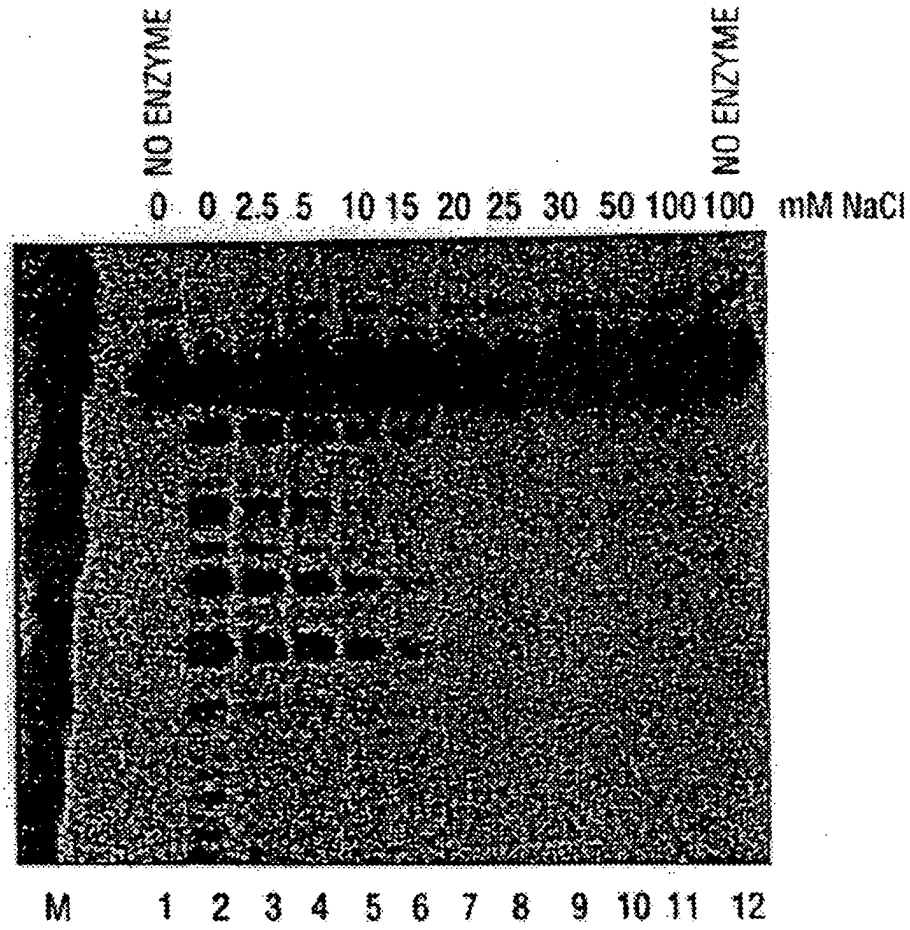

NO ENZYME
NO ENZYME
0 0 2.5 5 10 15 20 25 30 50 100 100 mM NaCl
M 1 2 3 4 5 6 7 8 9 10 11 12

FIG. 58

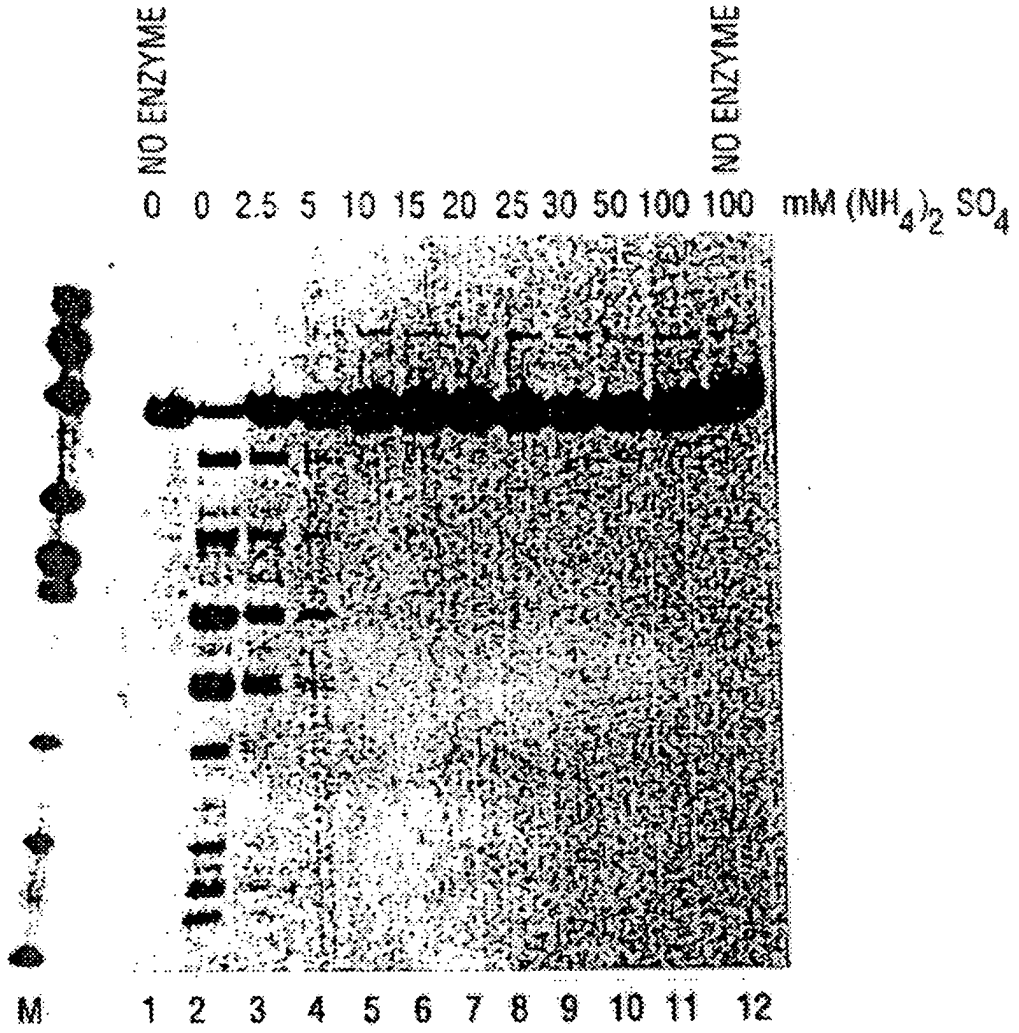

NO ENZYME
NO ENZYME
0 0 2.5 5 10 15 20 25 30 50 100 100 mM $(NH_4)_2$ $SO_4$
M 1 2 3 4 5 6 7 8 9 10 11 12

FIG. 59

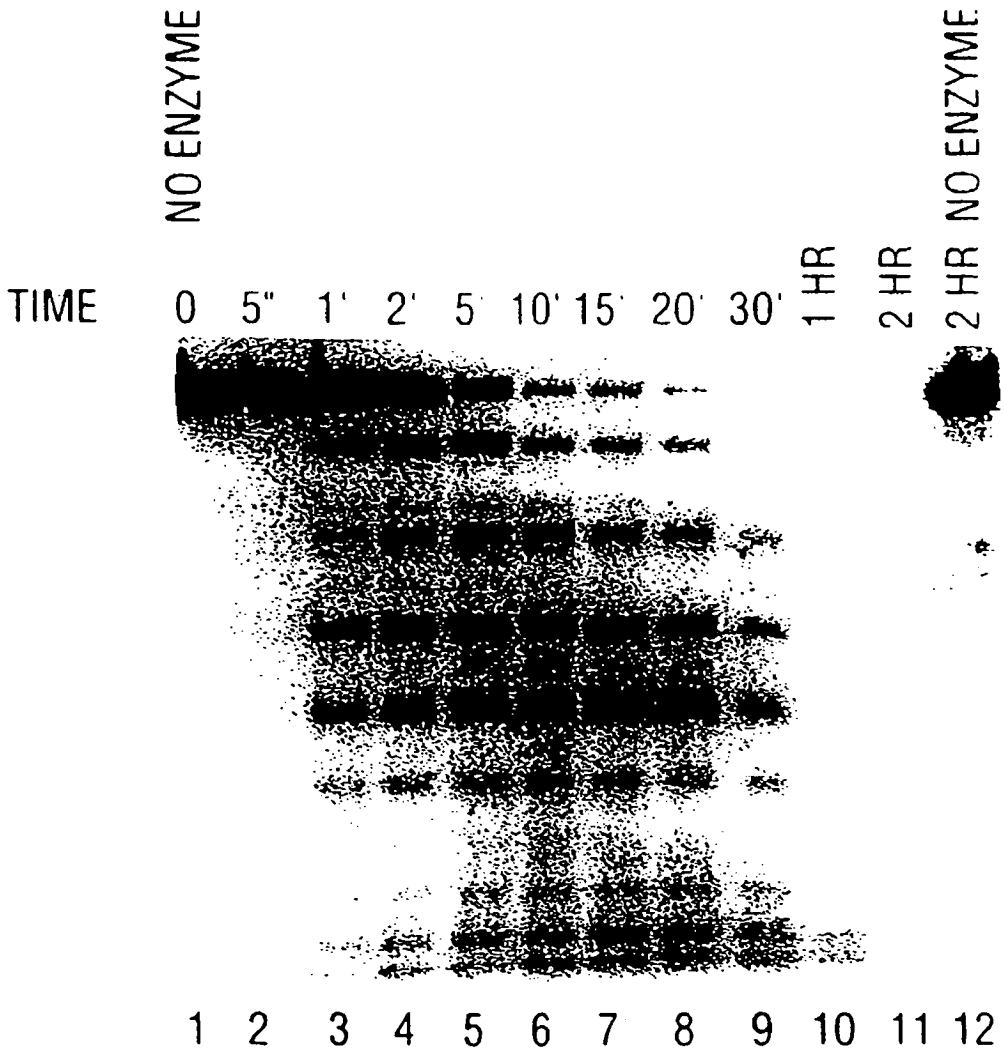

NO ENZYME
TIME
0
5"
1'
2'
5'
10'
15'
20'
30'
1 HR
2 HR
2 HR
NO ENZYME
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 60

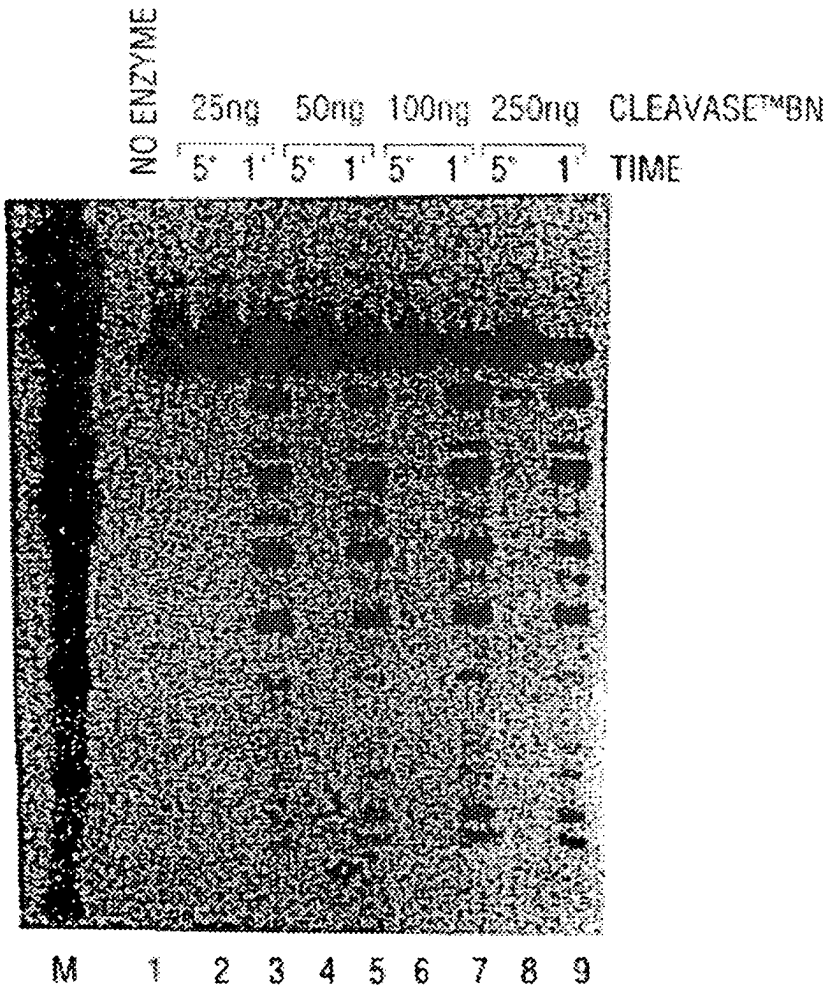

NO ENZYME
25ng
50ng
100ng
250ng
CLEAVASE™BN
5' 1' 5' 1' 5' 1' 5' 1'
TIME
M 1 2 3 4 5 6 7 8 9

FIG. 61

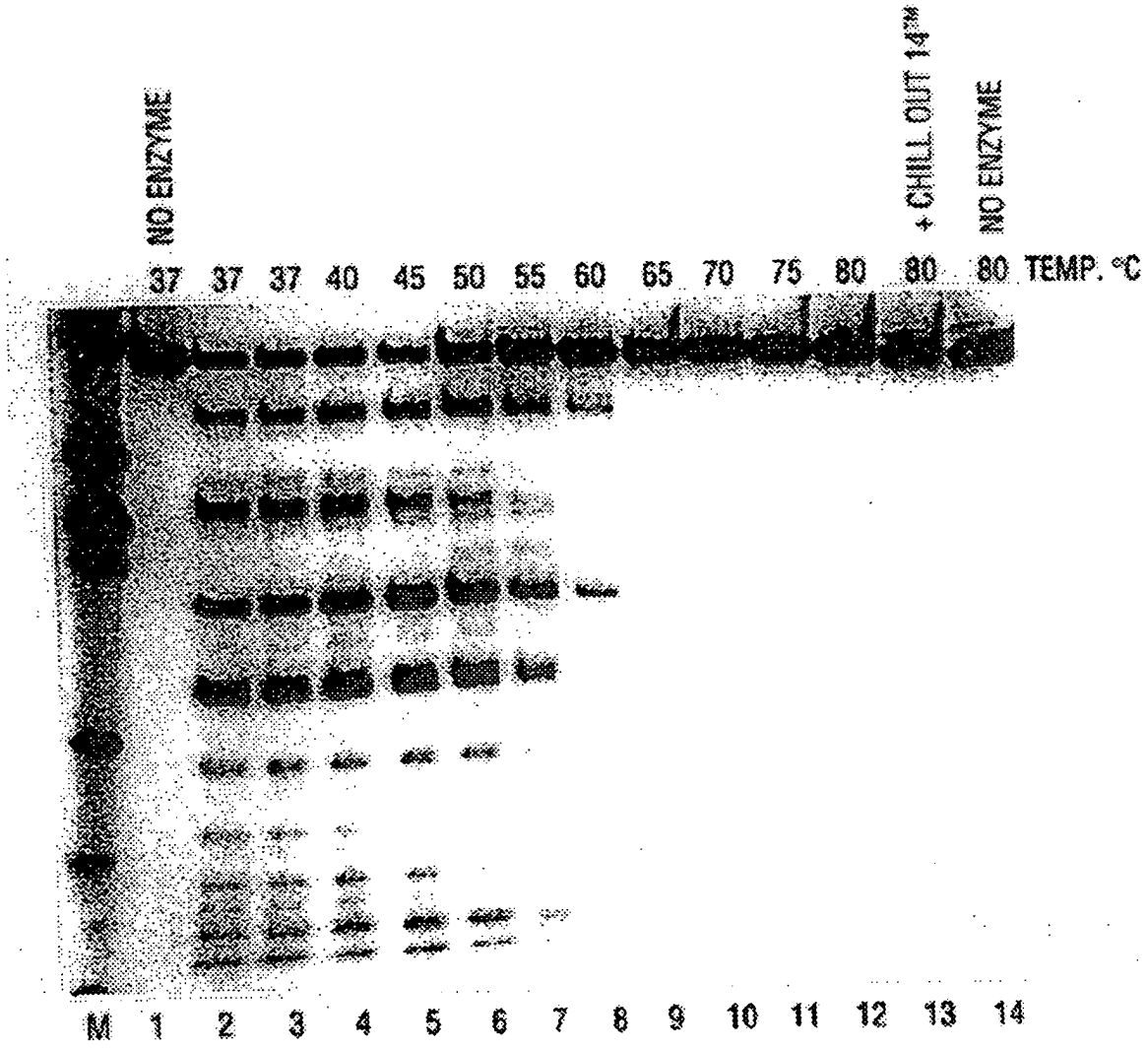

NO ENZYME
37 37 37 40 45 50 55 60 65 70 75 80 80 80 TEMP. °C
+ CHILL OUT 14™
NO ENZYME
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 62

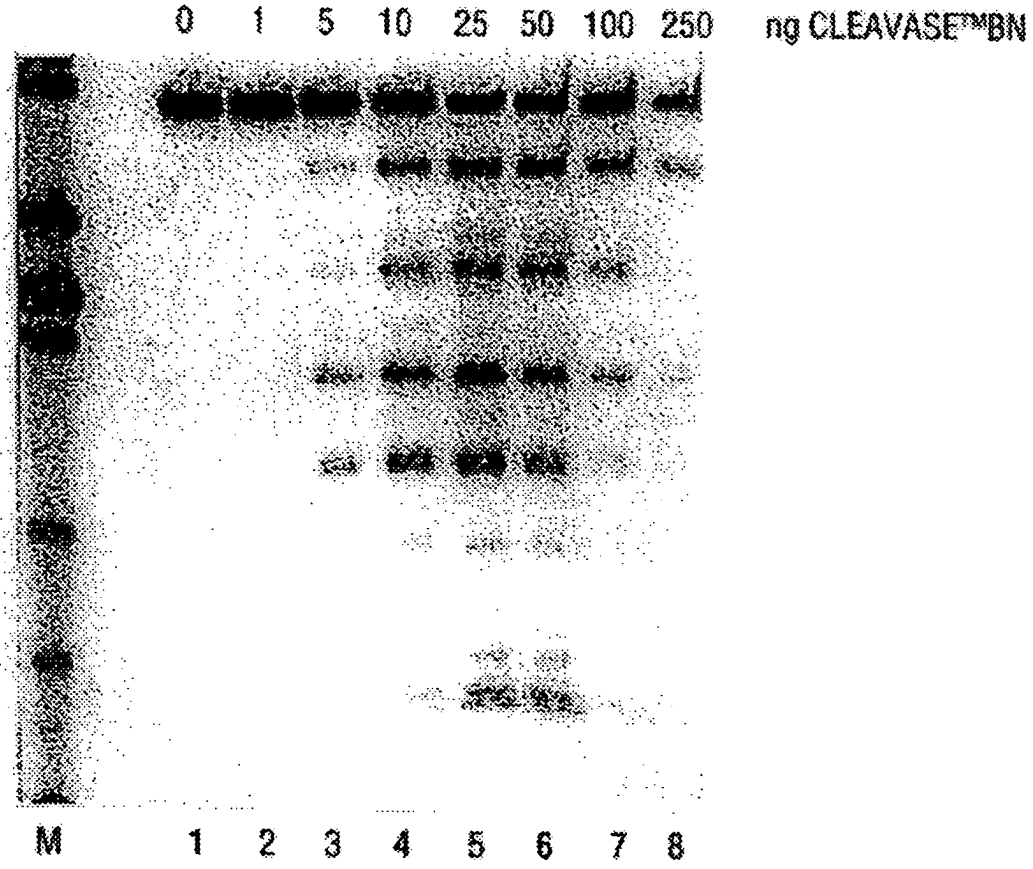



FIG. 63

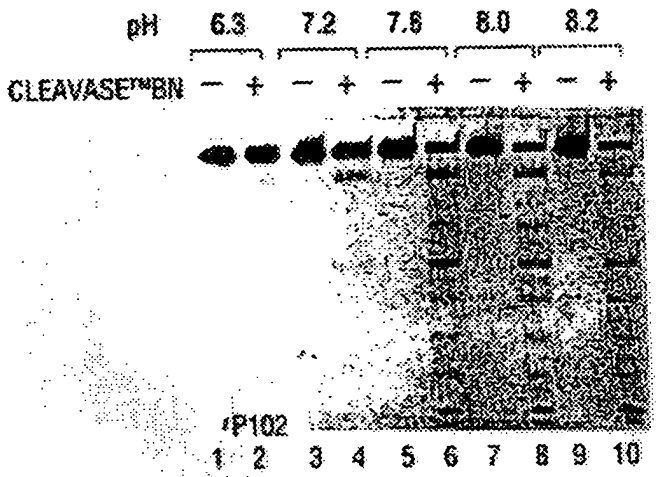



FIG. 64A

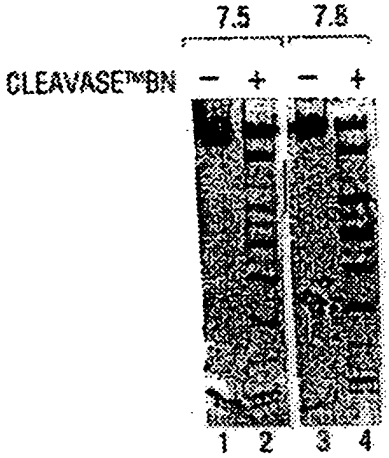



FIG. 64B

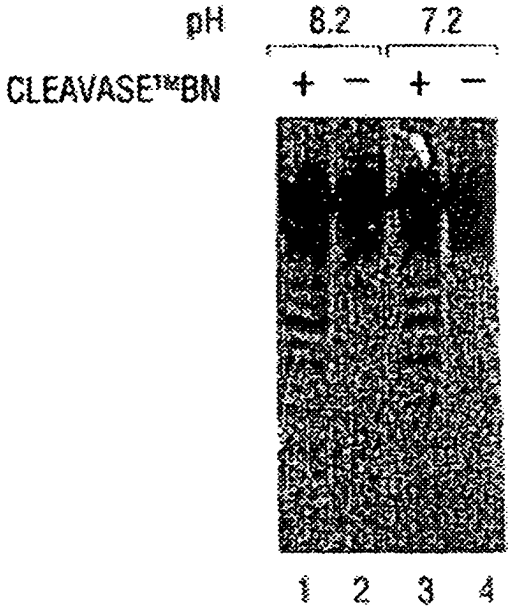



FIG. 65A

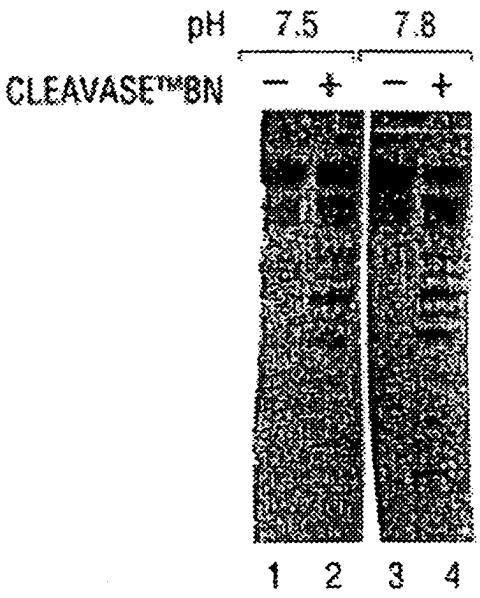



FIG. 65B

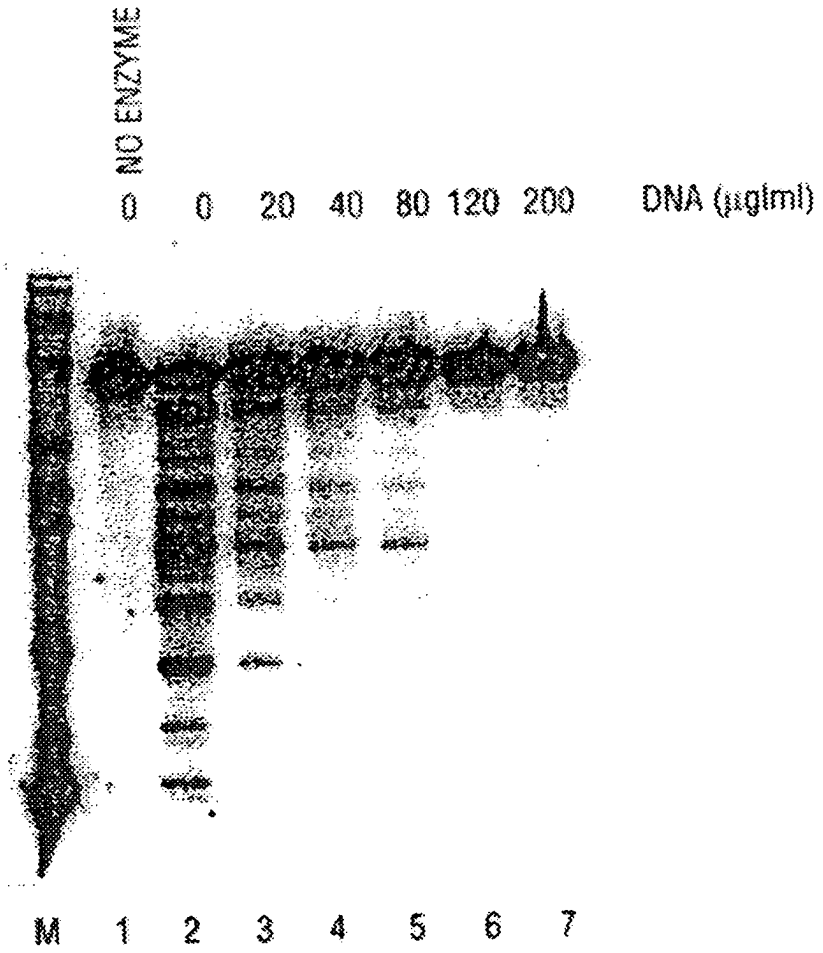



FIG. 66

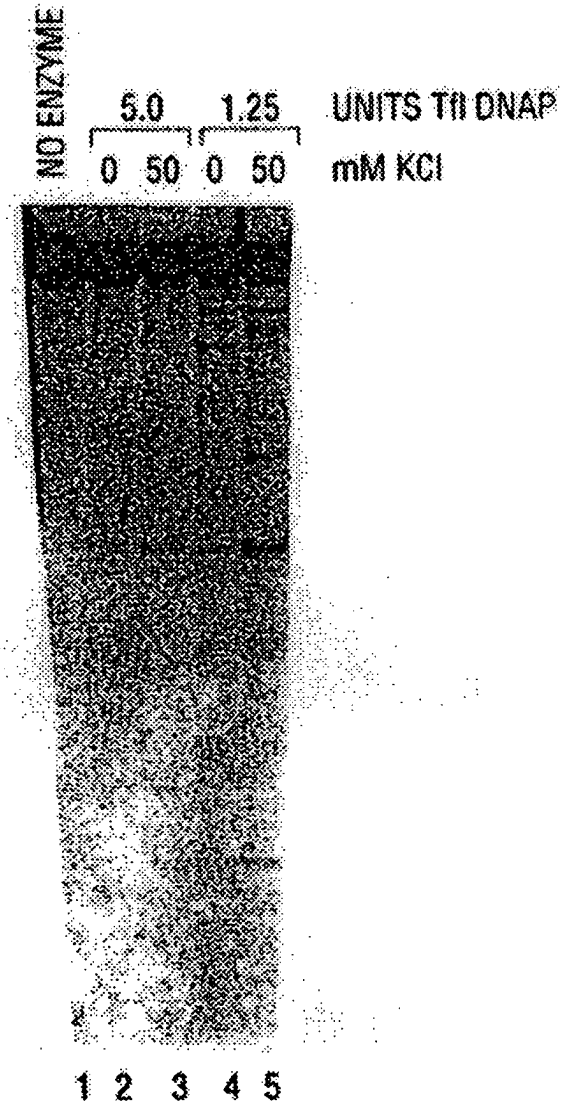



FIG. 67

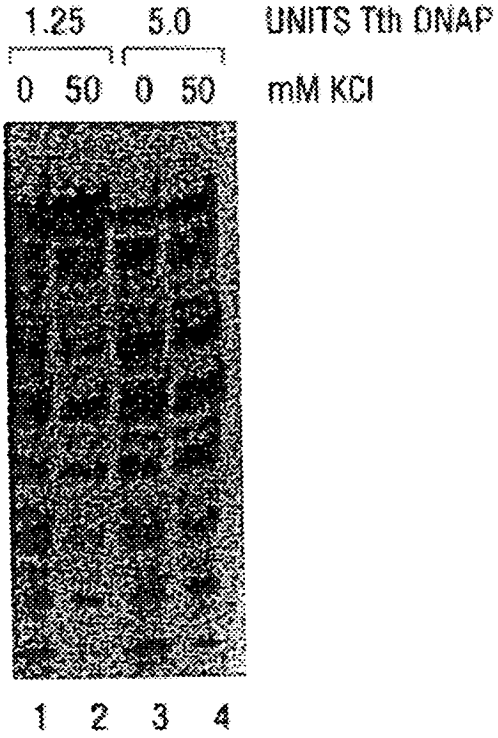



FIG. 68

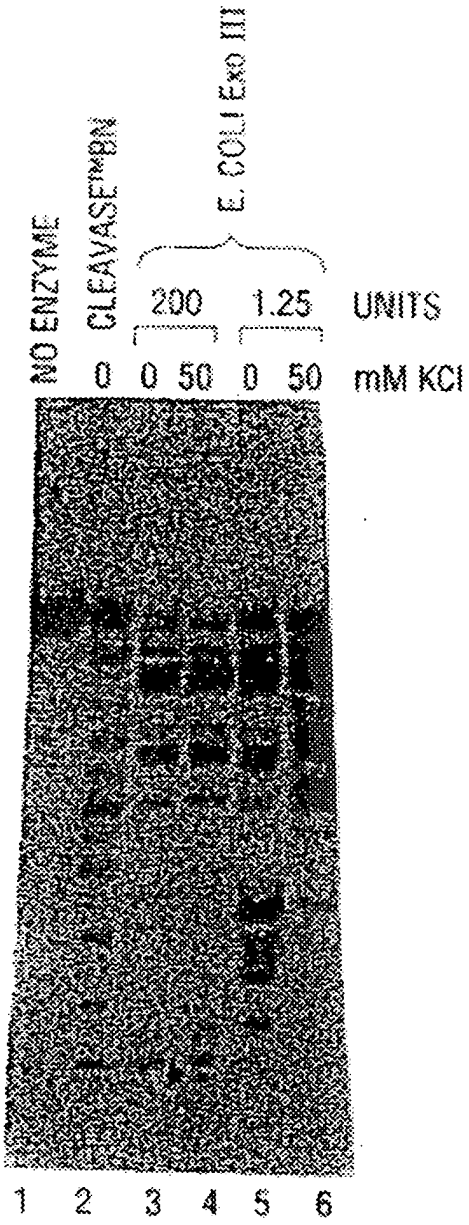

E. COLI Exo III
NO ENZYME
CLEAVASE™BN
200
1.25
UNITS
0 0 50 0 50
mM KCl
1 2 3 4 5 6

FIG. 69

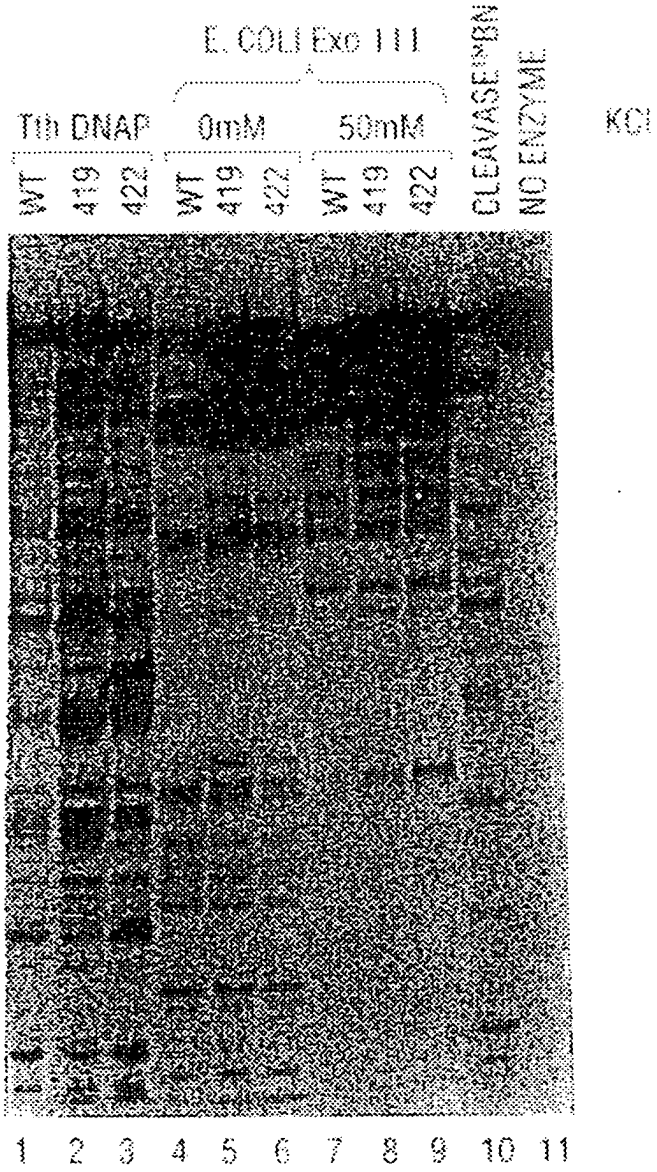

E. COLI Exo III
Tth DNAP
0mM
50mM
KCl
WT
419
422
WT
419
422
WT
419
422
CLEAVASE™BN
NO ENZYME
1 2 3 4 5 6 7 8 9 10 11

FIG. 70

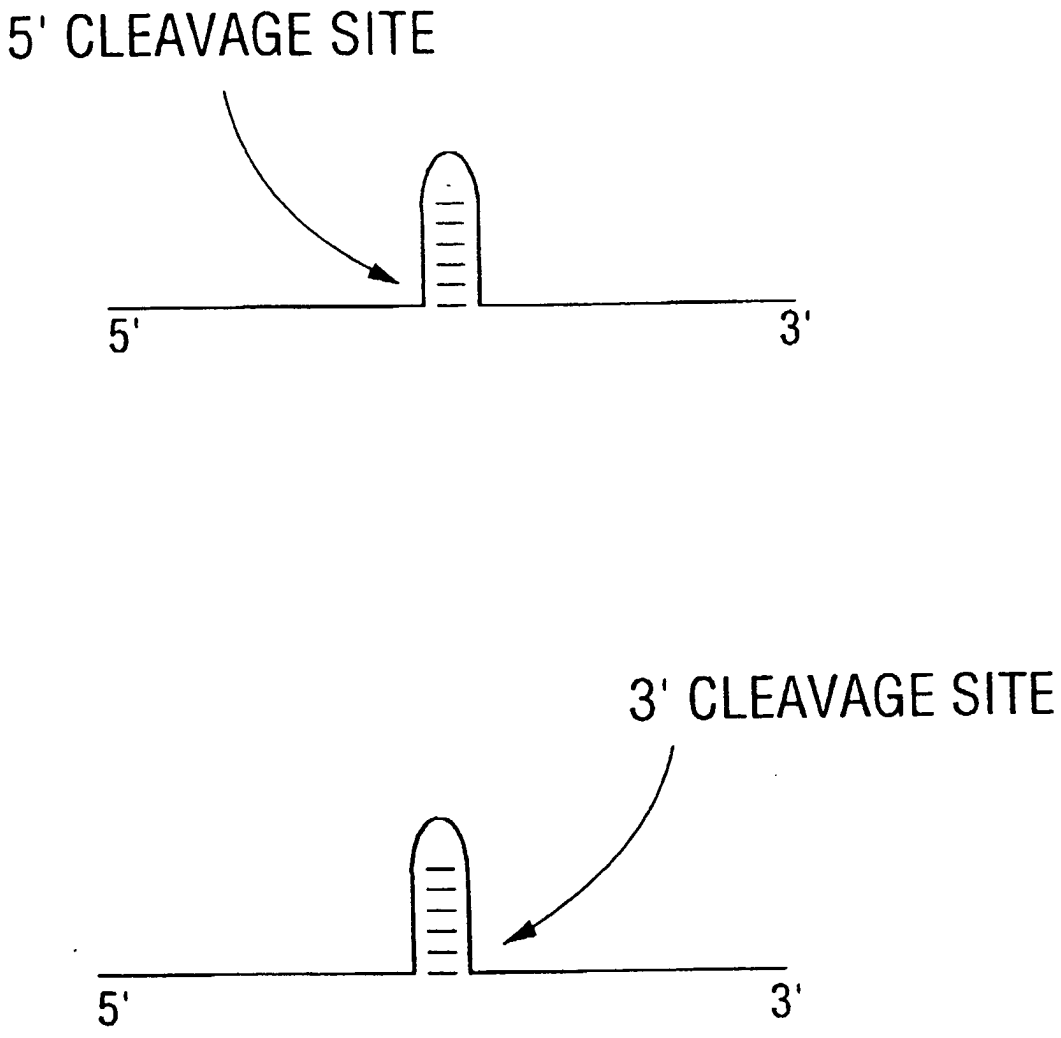



FIG. 71

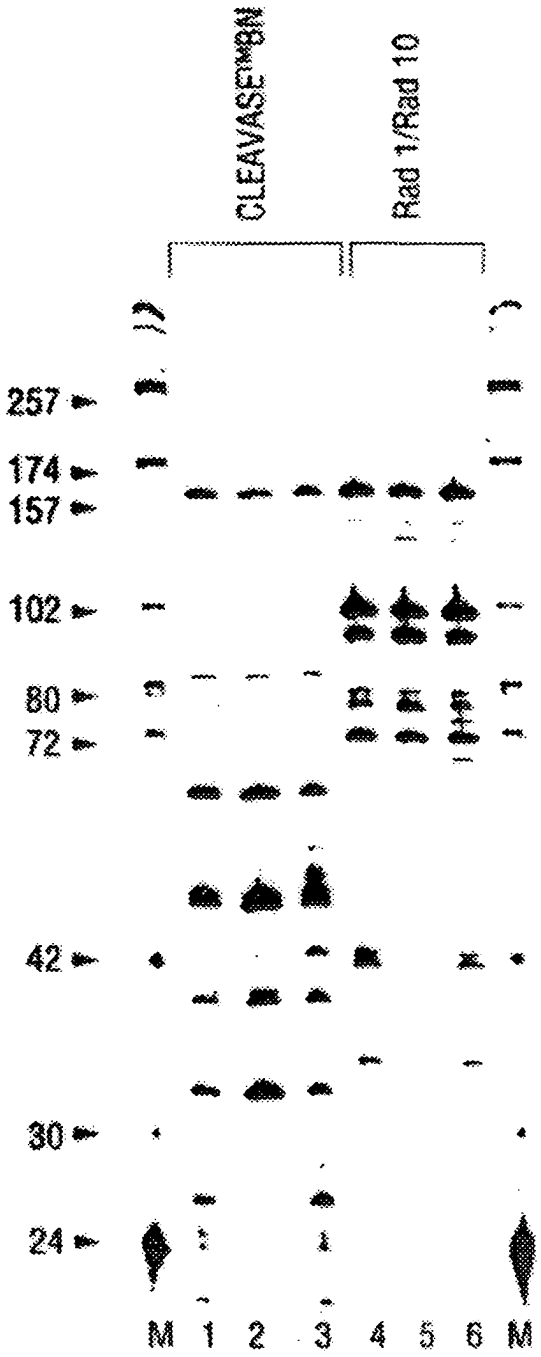



FIG. 72

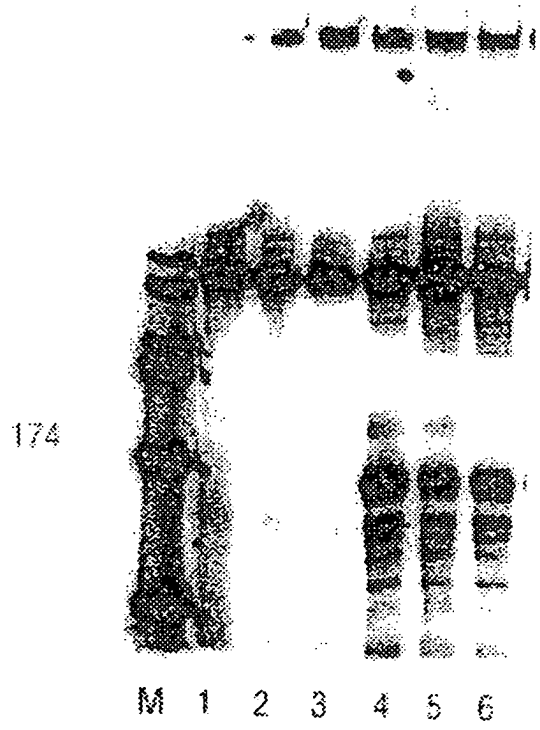



FIG. 73

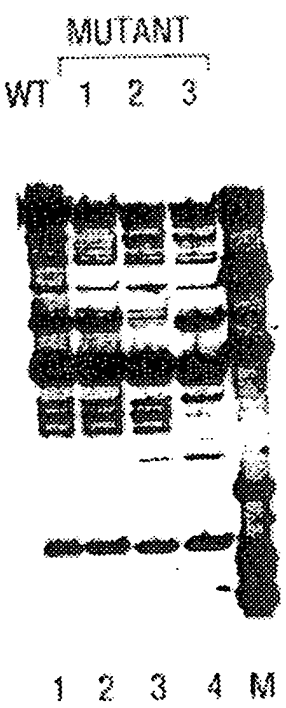



FIG. 74A

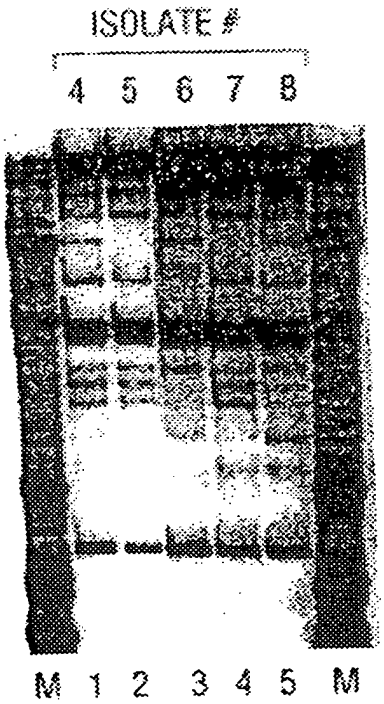



FIG. 74B

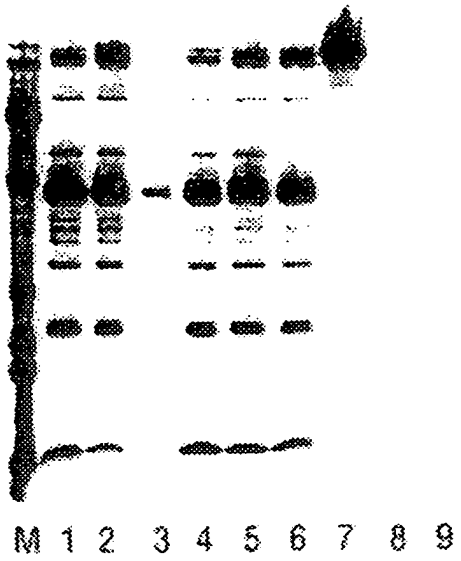



FIG. 75

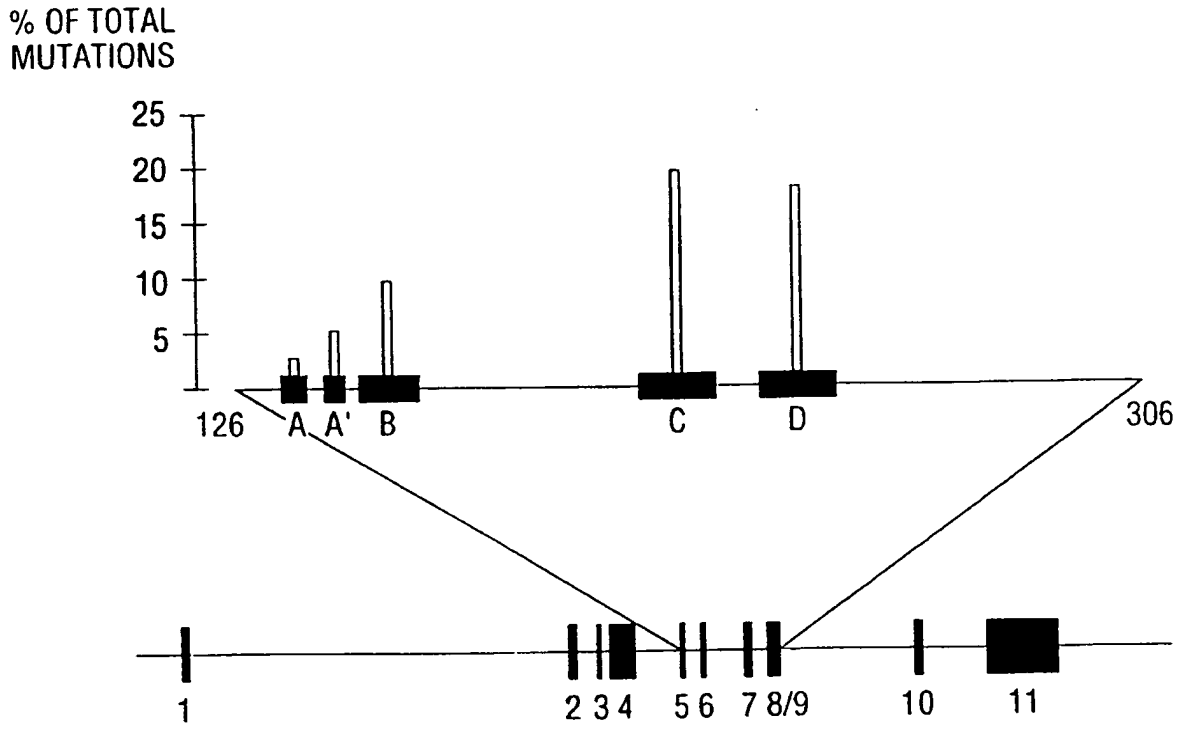



FIG. 76

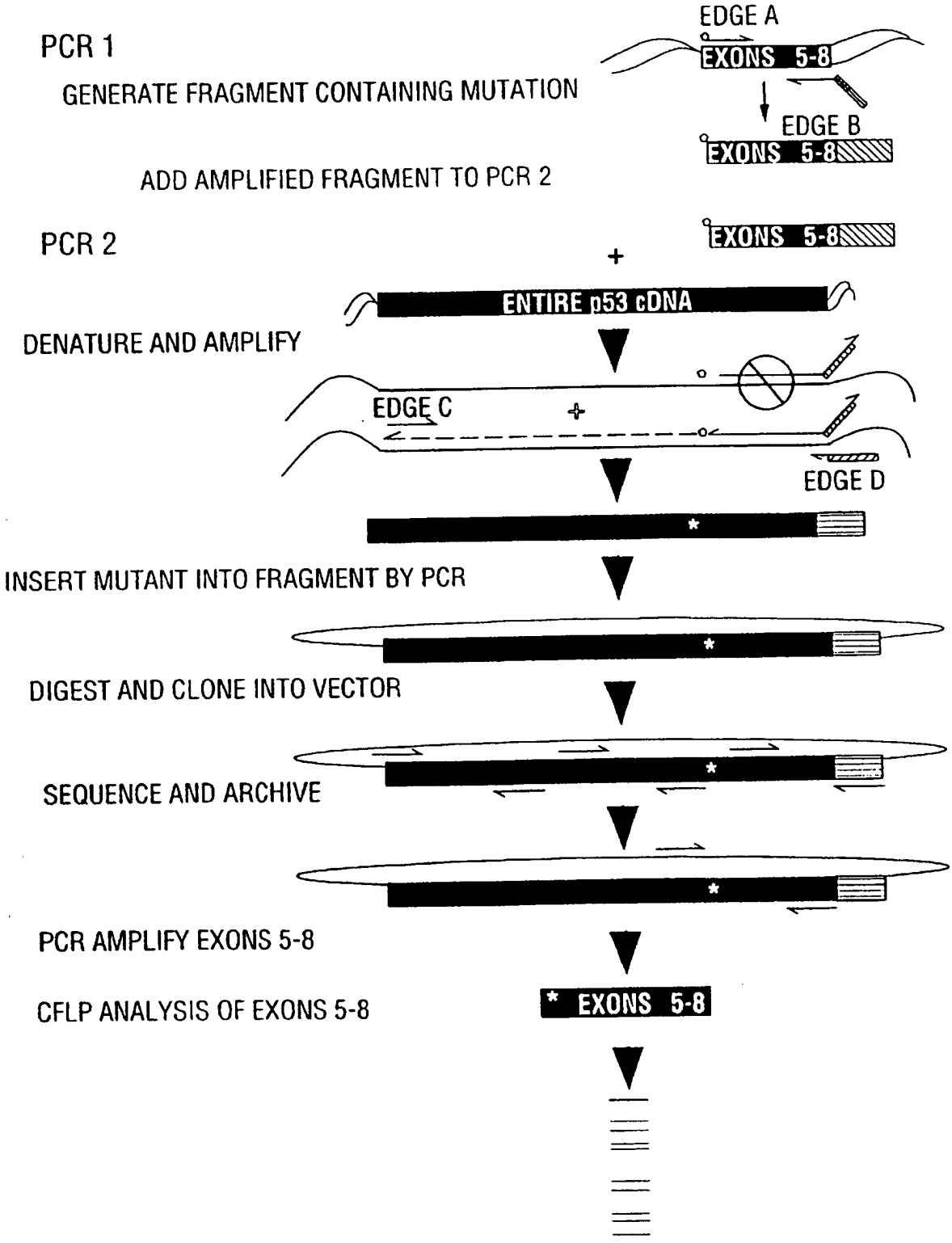

PCR 1
GENERATE FRAGMENT CONTAINING MUTATION
EDGE A
EXONS 5-8
EDGE B
EXONS 5-8
ADD AMPLIFIED FRAGMENT TO PCR 2
PCR 2
EXONS 5-8
+
ENTIRE p53 cDNA
DENATURE AND AMPLIFY
EDGE C
EDGE D
INSERT MUTANT INTO FRAGMENT BY PCR
DIGEST AND CLONE INTO VECTOR
SEQUENCE AND ARCHIVE
PCR AMPLIFY EXONS 5-8
CFLP ANALYSIS OF EXONS 5-8
* EXONS 5-8

FIG. 77

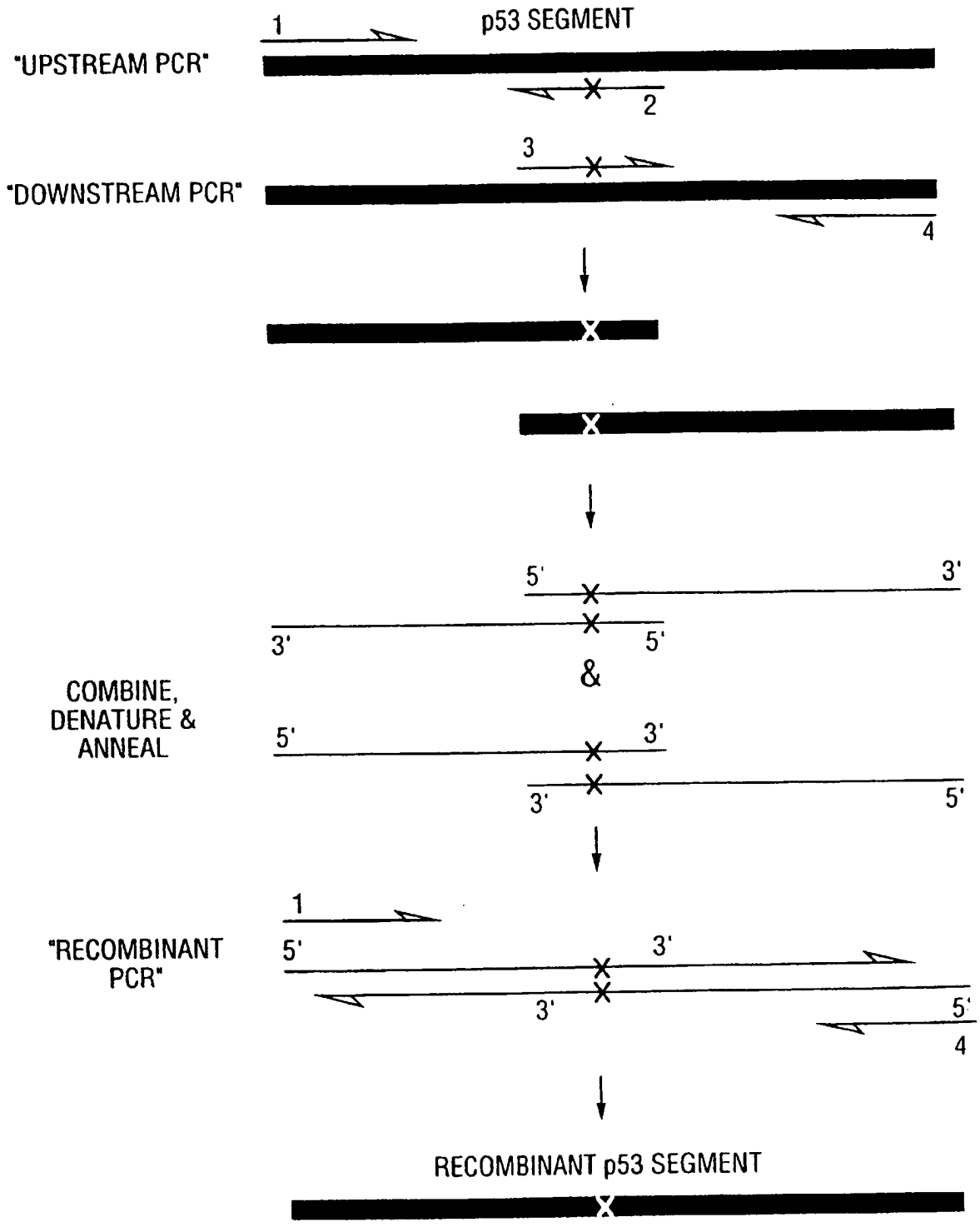

1
p53 SEGMENT
"UPSTREAM PCR"
2
3
"DOWNSTREAM PCR"
4
5'
3'
3'
5'
&
COMBINE,
DENATURE &
ANNEAL
5'
3'
3'
5'
1
"RECOMBINANT
PCR"
5'
3'
3'
5'
4
RECOMBINANT p53 SEGMENT

FIG. 78

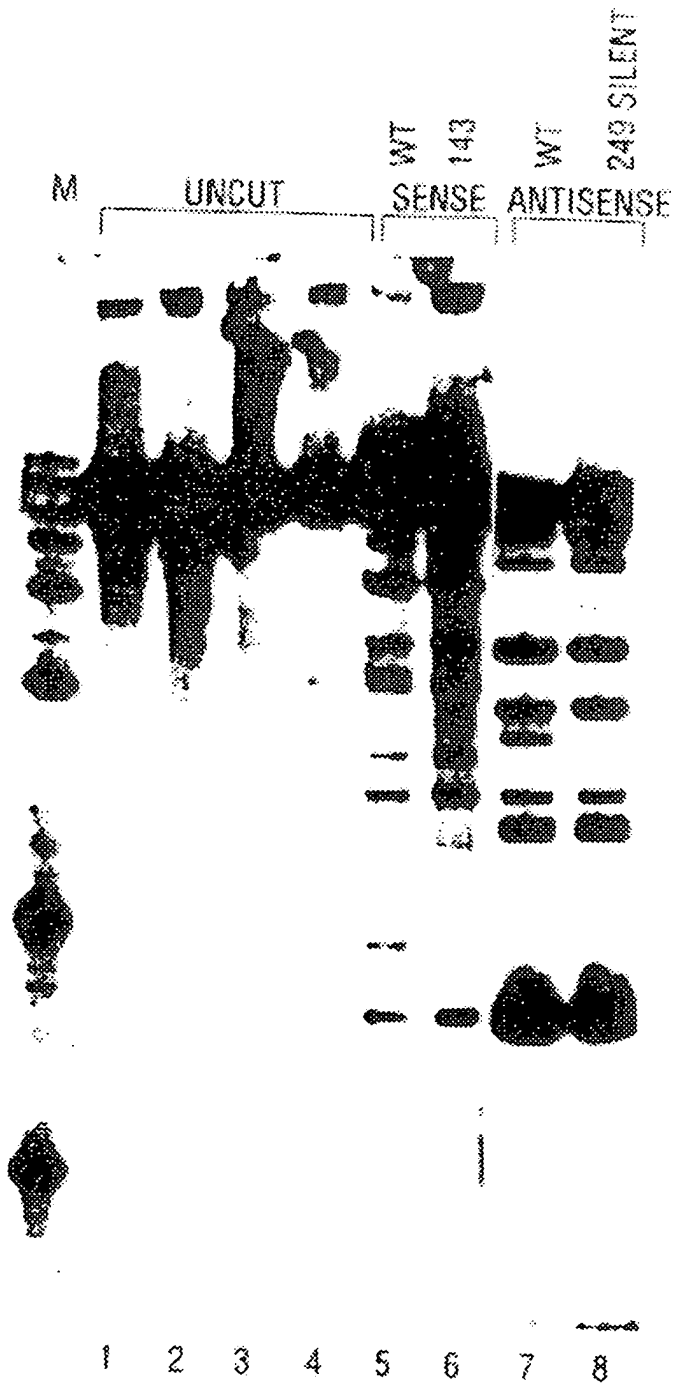



FIG. 79

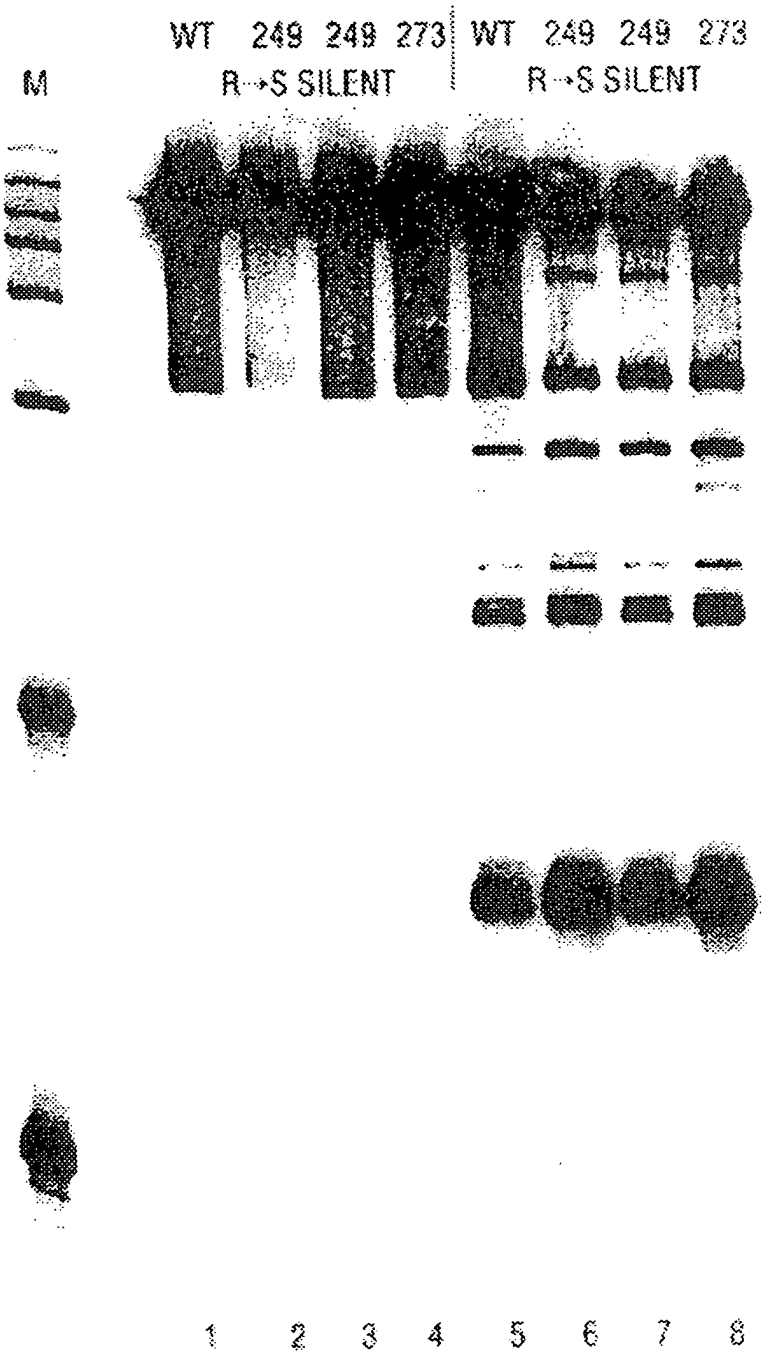



FIG. 80

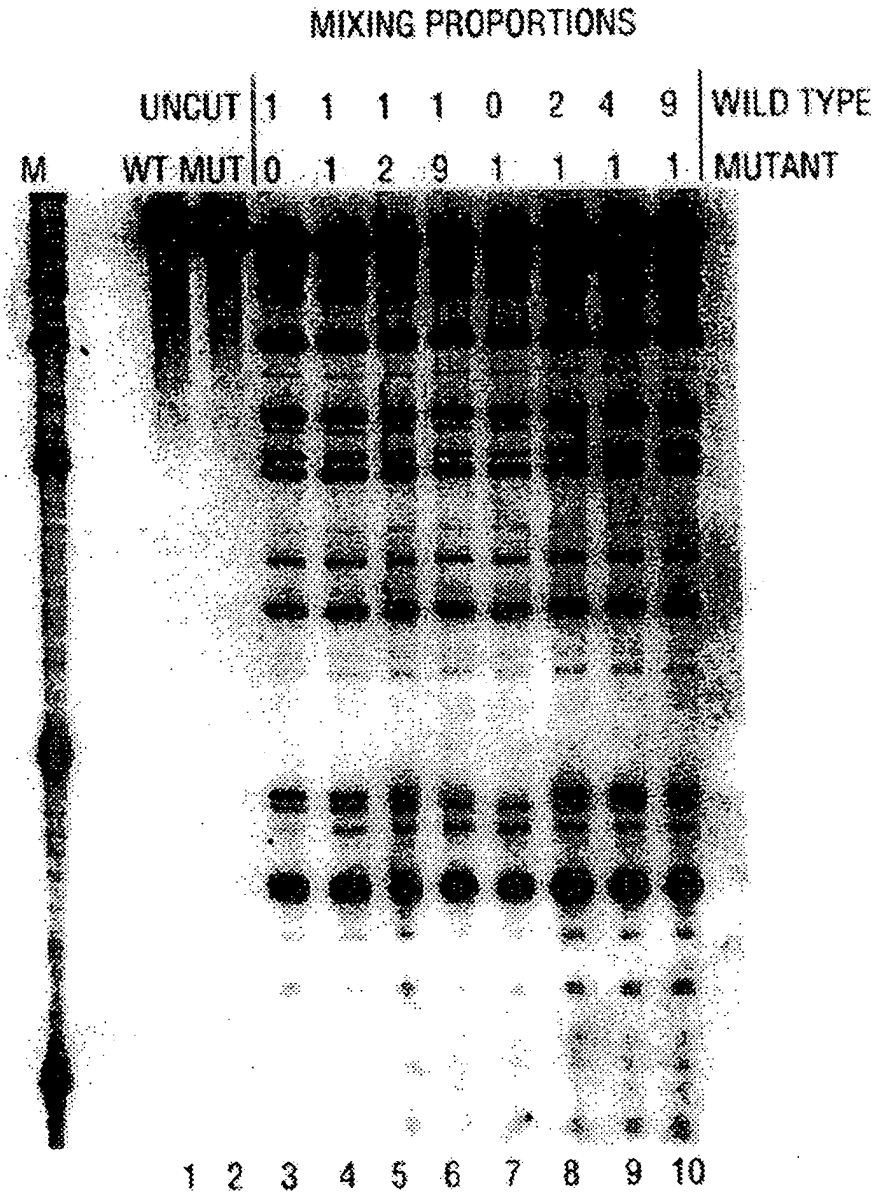

MIXING PROPORTIONS
UNCUT
1 1 1 1 0 2 4 9
WILD TYPE
M
WT MUT
0 1 2 9 1 1 1 1
MUTANT
1 2 3 4 5 6 7 8 9 10

FIG. 81

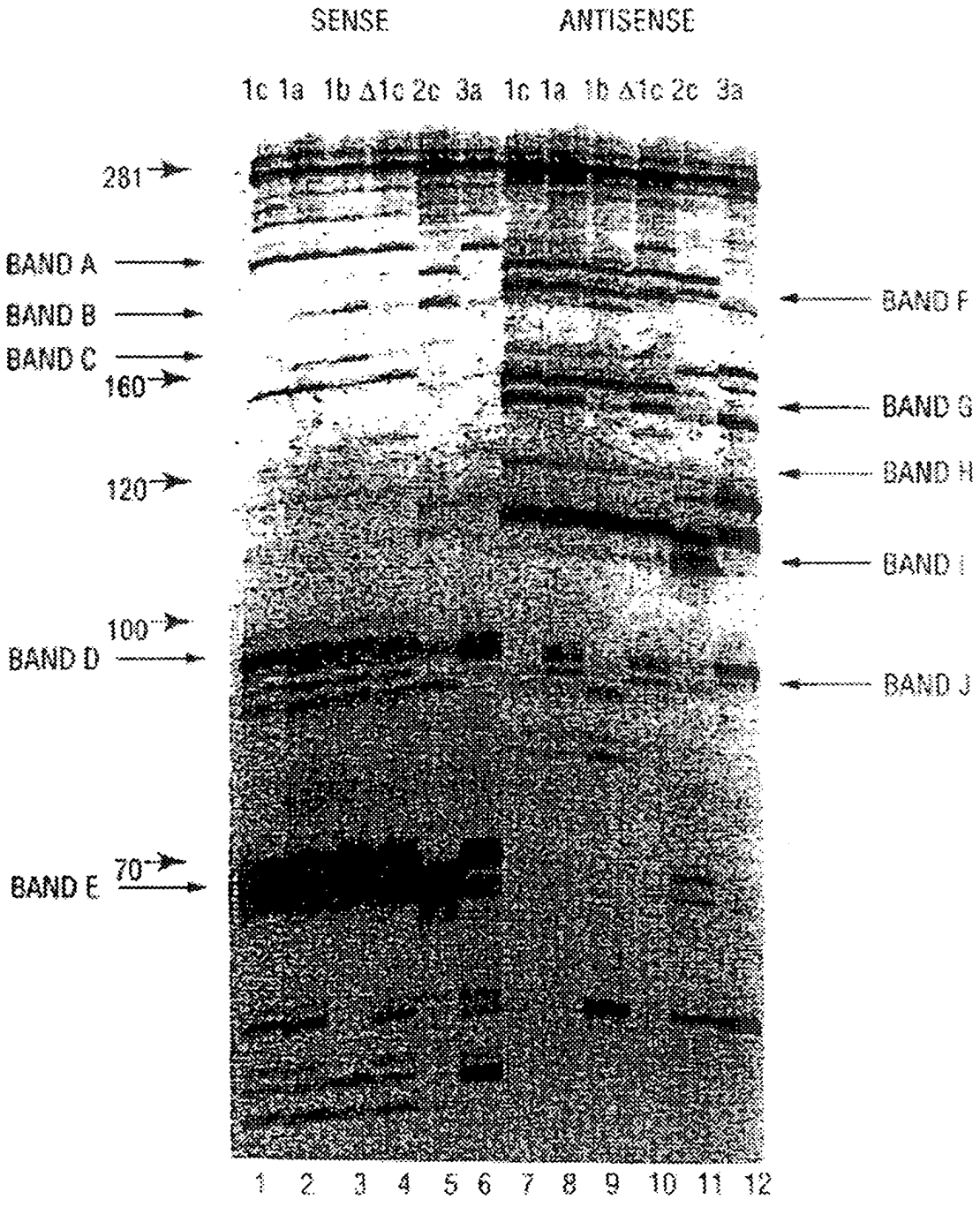

SENSE
ANTISENSE
1c 1a 1b Δ1c 2c 3a 1c 1a 1b Δ1c 2c 3a
281
BAND A
BAND B
BAND C
160
120
100
BAND D
70
BAND E
BAND F
BAND G
BAND H
BAND I
BAND J
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 83

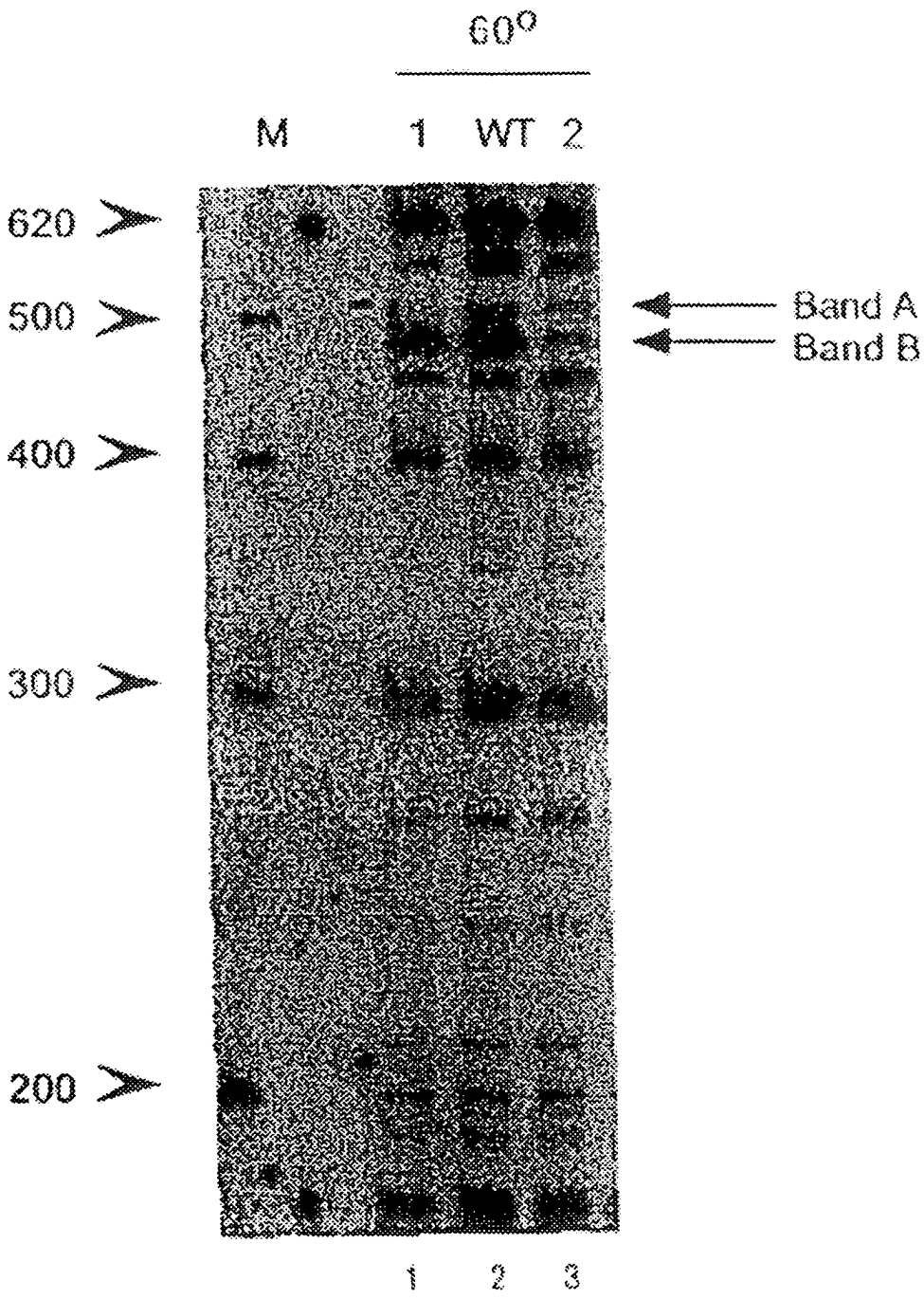



FIG. 84

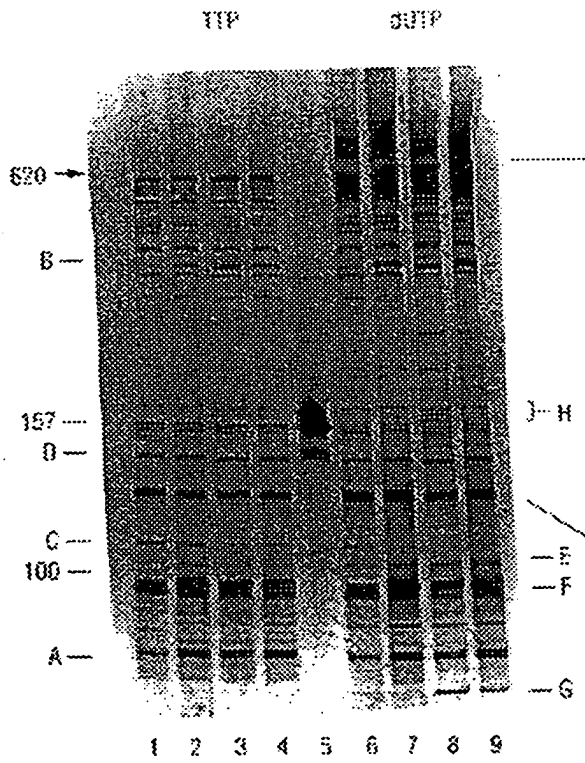



FIG. 85A

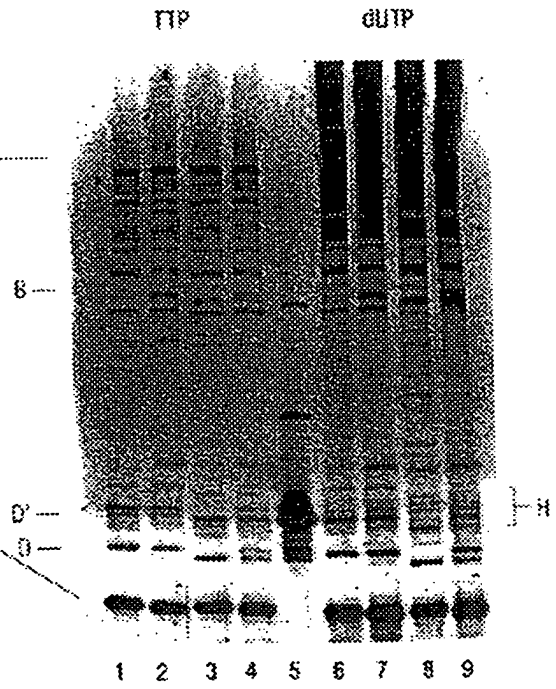



FIG. 85B

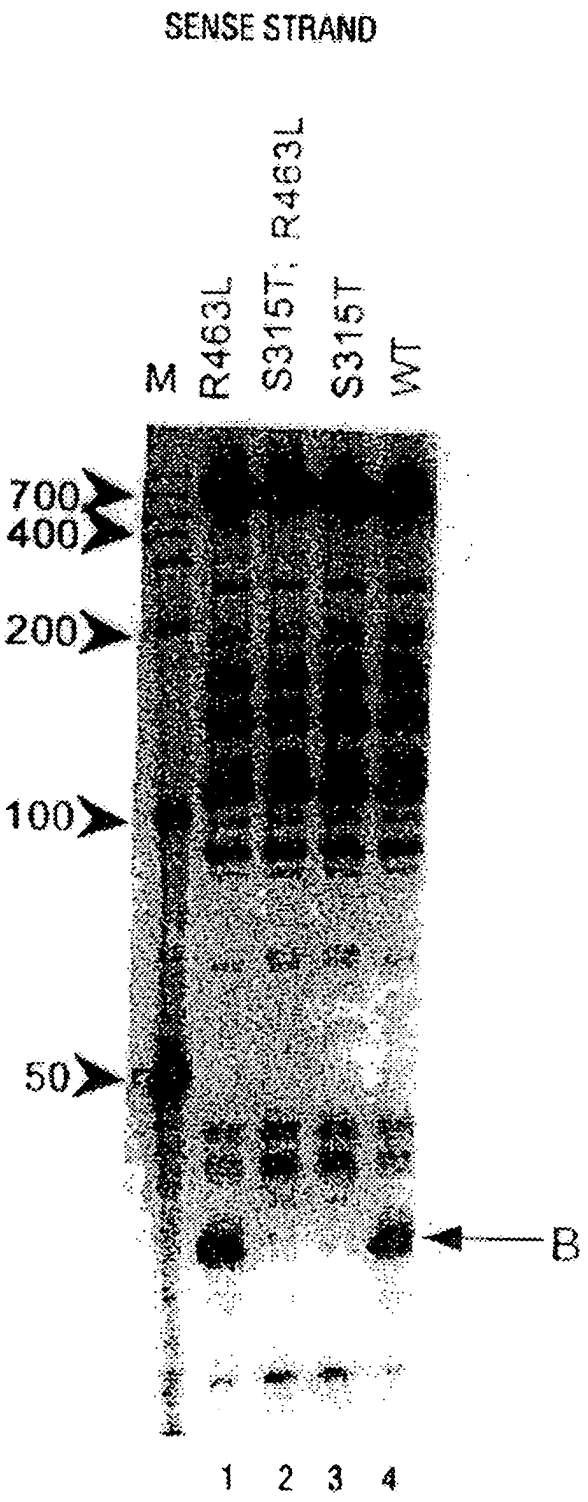

SENSE STRAND
M
R463L
S315T, R463L
S315T
WT
700
400
200
100
50
B
1
2
3
4

FIG. 86

```
HCVI.1 (SEQ ID NO:121)    1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG 50
HCV2.1 (SEQ ID NO:122)      CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV3.1 (SEQ ID NO:123)      CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV4.2 (SEQ ID NO:124)      CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV6.1 (SEQ ID NO:125)      CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTA
HCV7.1 (SEQ ID NO:126)      CTGTCTTCAC GCAGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTCGTG

HCV1.1                     51 CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC 100
HCV2.1                        CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV3.1                        CAGCCTCCAG GICCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV4.2                        CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV6.1                        CAGCCTCCAG GCCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV7.1                        CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC

HCV1.1                    101 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-AAA 150
HCV2.1                        GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV3.1                        GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV4.2                        GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC GTGGATGTAA
HCV6.1                        GGTGAGTACA CCGGAATTGC CGGGAAGACT GGGTCCTTTC TTGGAT-AAA
HCV7.1                        GGTGAGTACA CCGGAATCGC TGGGGTGACC GGGTCCTTTC TTGGAG-CAA
```

FIG. 82A

```
HCV1.1          151 CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG 200
HCV2.1              CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV3.1              CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCGAGA CTGCTAGCCG
HCV4.2              CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV6.1              CCCACTCIAT GCCCGGCCAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV7.1              CCCGCTCAAT ACCCAGAAAT TTGGGCGTGC CCCCGCGAGA ICACTAGCCG

HCVI.1          201 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCCT 250
HCV2.1              AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV3.1              AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV4.2              AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV6.1              AGTAGCGTTG GGTIGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV7.1              AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT

HCVI.1          251 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC 282
HCV2.1              GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV3.1              GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV4.2              GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV6.1              GCGAGTACCC CGGGAGGTCT CGTAGACCGT GC
HCV7.1              GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
```

**FIG. 82B**

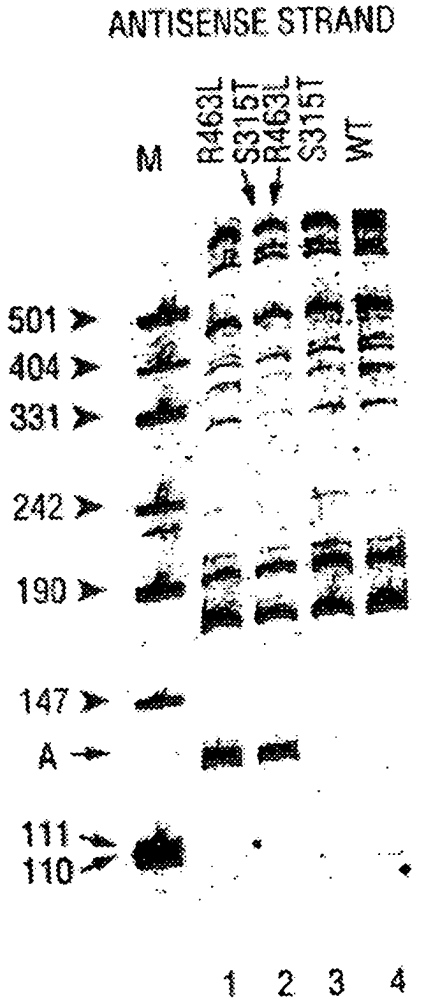

ANTISENSE STRAND
M
R463L
S315T
R463L
S315T
WT
501
404
331
242
190
147
A
111
110
1
2
3
4

FIG. 87

```
        10          20          30          40          50          60
       AGA GTTTGATCCT GGCTCAG                                          1638
AAATTGAAGA GTTTGATCAT GGCTCAGATT GAACGCTGGC GGCAGGCCTA ACACATGCAA
TTTAACTTCT CAAACTAGTA CCGAGTCTAA CTTGCGACCG CCGTCCGGAT TGTGTACGTT

        70          80          90          100         110         120
                                                   GGCGGAC GGGTGAGTAA  ER10
GTCGAACGGT AACAGGAAGA AGCTTGCTTC TTTGCTGACG AGTGGCGGAC GGGTGAGTAA
CAGCTTGCCA TTGTCCTTCT TCGAACGAAG AAACGACTGC TCACCGCCTG CCCACTCATT

       130         140         150         160         170         180
TGTCTGGGAA ACTGCCTGAT GGAGGGGGAT AACTACTGGA AACGGTAGCT AATACCGCAT
ACAGACCCTT TGACGGACTA CCTCCCCCTA TTGATGACCT TTGCCATCGA TTATGGCGTA

       190         200         210         220         230         240
AACGTCGCAA GACCAAAGAG GGGGACCTTC GGGCCTCTTG CCATCGGATG TGCCCAGATG
TTGCAGCGTT CTGGTTTCTC CCCCTGGAAG CCCGGAGAAC GGTAGCCTAC ACGGGTCTAC

       250         260         270         280         290         300
GGATTAGCTA GTAGGTGGGG TAACGGCTCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA
CCTAATCGAT CATCCACCCC ATTGCCGAGT GGATCCGCTG CTAGGGATCG ACCAGACTCT

       310         320         330         340         350         360
GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG
CCTACTGGTC GGTGTGACCT TGACTCTGTG CCAGGTCTGA GGATGCCCTC CGTCGTCACC
                                    TGA GGATGCCCTC CGTCGTC           1659
```

**FIG. 88A**

```
        370         380         390         400         410         420
GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG AAGAAGGCCT
CCTTATAACG TGTTACCCGC GTTCGGACTA CGTCGGTACG GCGCACATAC TTCTTCCGGA

        430         440         450         460         470         480
TCGGGTTGTA AAGTACTTTC AGCGGGGAGG AAGGGAGTAA AGTTAATACC TTTGCTCATT
AGCCCAACAT TTCATGAAAG TCGCCCCTCC TTCCCTCATT TCAATTATGG AAACGAGTAA

        490         500         510         520         530         540
GACGTTACCC GCAGAAGAAG CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG
CTGCAATGGG CGTCTTCTTC GTGGCCGATT GAGGCACGGT CGTCGGCGCC ATTATGCCTC

        550         560         570         580         590         600
GGTGCAAGCG TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTT TGTTAAGTCA
CCACGTTCGC AATTAGCCTT AATGACCCGC ATTTCGCGTG CGTCCGCCAA ACAATTCAGT

        610         620         630         640         650         660
GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGCATCTGA TACTGGCAAG CTTGAGTCTC
CTACACTTTA GGGGCCCGAG TTGGACCCTT GACGTAGACT ATGACCGTTC GAACTCAGAG

        670         680         690         700         710         720
GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG TAGAGATCTC GAGGAATACC
CATCTCCCCC CATCTTAAGG TCCACATCGC CACTTTACGC ATCTCTAGAC CTCCTTATGG

        730         740         750         760         770         780
GGTGGCGAAG GCGGCCCCCT GGACGAAGAC TGACGCTCAG GTGCGAAAGC GTGGGGAGCA
CCACCGCTTC CGCCGGGGGA CCTGCTTCTG ACTGCGAGTC CACGCTTTCG CACCCCTCGT
```

**FIG. 88B**

```
       790         800         810         820         830         840
AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTGCC
TTGTCCTAAT CTATGGGACC ATCAGGTGCG GCATTTGCTA CAGCTGAACC TCCAACACGG

       850         860         870         880         890         900
CTTGAGGCGT GGCTTCCGGA GCTAACGCGT TAAGTCGACC GCCTGGGGAG TACGGCCGCA
GAACTCCGCA CCGAAGGCCT CGATTGCGCA ATTCAGCTGG CGGACCCCTC ATGCCGGCGT

       910         920         930         940         950         960
AGGTTAAAAC TCAAATGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT GTGGTTTAAT
TCCAATTTTG AGTTTACTTA ACTGCCCCCG GGCGTGTTCG CCACCTCGTA CACCAAATTA

       970         980         990        1000        1010        1020
TCGATGCAAC GCGAAGAACC TTACCTGGTC TTGACATCCA CGGAAGTTTT CAGAGATGAG
AGCTACGTTG CGCTTCTTGG AATGGACCAG AACTGTAGGT GCCTTCAAAA GTCTCTACTC

      1030        1040        1050        1060        1070        1080
AATGTGCCTT CGGGAACCGT GAGACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTTGTGA
TTACACGGAA GCCCTTGGCA CTCTGTCCAC GACGTACCGA CAGCAGTCGA GCACAACACT

      1090        1100        1110        1120        1130        1140
                    GC AACGAGCGCA ACCC                                    SB-1

AATGTTGGGT TAAGTCCCGC AACGAGCGCA ACCCTTATCC TTTGTTGCCA GCGGTCCGGC
TTACAACCCA ATTCAGGGCG TTGCTCGCGT TGGGAATAGG AAACAACGGT CGCCAGGCCG

      1150        1160        1170        1180        1190        1200
                                                      ATG ACGTCAAGTC  SB-3
                                                      ATG ACGTCAAGTC  SB-4
CGGGAACTCA AAGGAGACTG CCAGTGATAA ACTGGAGGAA GGTGGGGATG ACGTCAAGTC
GCCCTTGAGT TTCCTCTGAC GGTCACTATT TGACCTCCTT CCACCCCTAC TGCAGTTCAG
```

**FIG. 88C**

```
      1210        1220        1230        1240        1250        1260
ATCATGGCCC TTA                                                          SB-3
ATCATGGCCC TTACGA                                                       SB-4
ATCATGGCCC TTACGACCAG GGCTACACAC GTGCTACAAT GGCGCATACA AAGAGAAGCG
TAGTACCGGG AATGCTGGTC CCGATGTGTG CACGATGTTA CCGCGTATGT TTCTCTTCGC

      1270        1280        1290        1300        1310        1320
ACCTCGCGAG AGCAAGCGGA CCTCATAAAG TGCGTCGTAG TCCGGATTGG AGTCTGCAAC
TGGAGCGCTC TCGTTCGCCT GGAGTATTTC ACGCAGCATC AGGCCTAACC TCAGACGTTG

      1330        1340        1350        1360        1370        1380
TCGACTCCAT GAAGTCGGAA TCGCTAGTAA TCGTGGATCA GAATGCCACG GTGAATACGT
AGCTGAGGTA CTTCAGCCTT AGCGATCATT AGCACCTAGT CTTACGGTGC CACTTATGCA
                                                    GC CACTTATGCA       1743

      1390        1400        1410        1420        1430        1440
TCCCGGGCCT TGTACACACC GCCCGTCACA CCATGGGAGT GGGTTGCAAA AGAAGTAGGT
AGGGCCCGGA ACATGTGTGG CGGGCAGTGT GGTACCCTCA CCCAACGTTT TCTTCATCCA
                                                                        1743
AGGGCCCGGA ACATG

      1450        1460        1470        1480        1490        1500
AGCTTAACCT TCGGGAGGGC GCTTACCACT TTGTGATTCA TGACTGGGGT GAAGTCGTAA
TCGAATTGGA AGCCCTCCCG CGAATGGTGA AACACTAAGT ACTGACCCCA CTTCAGCATT

      1510        1520        1530        1540        1550
CAAGGTAACC GTAGGGGAAC CTGCGGTTGG ATCACCTCCT TA.......
GTTCCATTGG CATCCCCTTG GACGCCAACC TAGTGGAGGA AT.......
```

**FIG. 88D**

```
1638 (SEQ ID NO:151)                    AGAGTTTGATCCTGGCTCAG
E.colirrsE(SEQ ID NO:158)0 ...AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCA
Cam.jejun5(SEQ ID NO:159)0 ~TTTTTATGGAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCGTGCCTAATACATGCA
Stp.aureus(SEQ ID NO:160)0 ..TTTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA

ER10 (SEQ ID NO:152)                                                    GGCGGACGGG
E.colirrsE              60 AGTCGAACGGTAACAG----GAAGAAGCTTGCTTCTTT----GCTGACGAGTGGCGGACGGG
Cam.jejunS              62 AGTCGAACGAT-----GAAGCTTCTAGCTTGCTAGAAGTGGA-----TTAGTGGCGCACGGG
Stp.aureus              61 AGTCGAGCGAA-----CGGACGAGAAGCTTGCTTCTCTGATG----TT-AGCGGCGGACGGG

ER10                       TGAGTAA
E.colirrsE             114 TGAGTAATGTCTGGGA-AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA
Cam.jejun5             114 TGAGTAAGGTATAGTTAATCTGCCCTACACAAGAGGACAACAGTTGGAAACGACTGCTAATA
Stp.aureus             113 TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATA

E.colirrsE             175 CCGCATAAC-------GTCGCAAGAC--------CAAAGAGGGGGACCTTCG-GGCCTCTTG
Cam.jejun5             176 CTCTATACTCCTGCTTAACACAAGTTGAGTAGG-GAAAG---------TTTTT-------CG
Stp.aureus             175 CCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGT----CTT----GCTGTCA

E.colirrsE             221 CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGA
Cam.jejun5             221 GTGTAGGATGAGACTATATAGTATCAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCTATGA
Stp.aureus             229 CTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGA

E.colirrsE             283 TCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
Cam.jejun5             283 CGCTTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA
Stp.aureus             291 TACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
1659(COMPL)                                                             ACTCCTA
```

FIG. 89A

```
E.colirrsE        345 CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
Cam.jejun5        345 CGGGAGGCAGCAGTAGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTG
Stp.aureus        353 CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTG
1659(COMPL)           CGGGAGGCAGCAG

E.colirrsE        407 TATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA-GGGAGTAAAGTTAAT
Cam.jejun5        407 GAGGATGACACTTTTCGGAGCGTAAACTCCTTTTCTTAGGGAAG ----------AATT
Stp.aureus        415 AGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAAC

E.colirrsE        468 ACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Cam.jejun5        455 C----------TGACGGTACCTAAGGAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Stp.aureus        476 -TGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG
```

FIG. 89B

```
E.colirrsE   530 GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTT
Cam.jejun5   506 GTAATACGGAGGGTGCAAGCGTTACTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGATT
Stp.aureus   538 GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT

E.colirrsE   592 GTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTT
Cam.jejun5   568 ATCAAGTCTCTTGTGAAATCTAATGGCTTAACCATTAAACTGCTTGGGAAACTGATAGTCTA
Stp.aureus   600 TTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT

E.colirrsE   654 GAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
Cam.jejun5   630 GAGTGAGGGAGAGGCAGATGGAATTGGTGGTGTAGGGGTAAAATCCGTAGATATCACCAAGA
Stp-aureus   662 GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGA

E.colirrsE   716 ATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
Cam.jejun5   692 ATACCCATTGCGAAGGCGATCTGCTGGAACTCAACTGACGCTAAGGCGCGAAAGCGTGGGGA
Stp.aureus   724 ACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGA

E.colirrsE   778 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGC
Cam.jejun5   754 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTACACTAGTTGTTGGGGT
Stp.aureus   786 TCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGG
```

FIG. 89C

```
E.colirrsE   840 C-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGC
Cam.jejun5   816 G-CTAGT-CATCTCAGTAATGCAGCTAACGCATTAAGTGTACCGCCTGGGGAGTACGGTCGC
Stp.aureus   848 GT-TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGC

E.colirrsE   900 AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Cam.jejun5   876 AAGATTAAAACTCAAAGGAATAGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Stp.aureus   909 AAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT

E.colirrsE   962 CGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAAT
Cam.jejun5   938 CGAAGATACGCGAAGAACCTTACCTGGGCTTGATATCCTAAGAACCTTTTAGAGATAAGAGG
Stp.aureus   971 CGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCC

E.colirrsE  1024 GTG--CCTTCGGG--AA-CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
Cam.jejun5  1000 GTGCTAGCTTGCTAGAA-CTTAGAGACAGGTGCTGCACGGCTGTCGTCAGCTCGTGTCGTGA
Stp.aureus  1033 TTCC-CCTTCGGG--GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA

SB-1                                    GCAACGAGCGCAACCC
E.colirrsE  1081 AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGG-CC
Cam.jejun5  1061 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTATTTAGTTGCTAACGGTTCGG-CC
Stp.aureus  1092 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCA-TTAAGT-T
```

**FIG. 89D**

```
SB-3 (SEQ ID NO:157)                                                    ATGACGTCAAGTCATC
SB-4 (SEQ ID NO:154)                                                    ATGACGTCAAGTCATC
E.colirrsE   1142 GGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATC
Cam.jejun5   1122 GAGCACTCTAAATAGACTGCCTTCG-TAAGGAGGAGGAAGGTGTGGACGACGTCAAGTCATC
Stp.aureus   1152 GGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC

SB-3                ATGGCCCTTA
SB-4                ATGGCCCTTACGA
E.colirrsE   1204 ATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTC
Cam.jejun5   1183 ATGGCCCTTATGCCCAGGGCGACACACGTGCTACAATGGCATATAGAATGAGACGCAATACC
Stp.aureus   1214 ATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACC

E.colirrsE   1266 GCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
Cam.jejun5   1245 GCGAGGTGGAG-CAAATCTATAAAATATGTCCCAGTTCGGATTGTTCTCTGCAACTCGAGAG
Stp.aureus   1276 GCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTA

E.colirrsE   1328 CATGAAGTCGGAATCGCTAGTAATCGTGGATCAGA-ATGCCACGGTGAATACGTTCCCGGGC
Cam.jejun5   1306 CATGAAGCCGGAATCGCTAGTAATCGTAGATCAGCCATGCTACGGTGAATACGTTCCCGGGT
Stp.aureus   1338 CATGAAGCTGGAATCGCTAGTAATCGTAGATCAGC-ATGCTACGGTGAATACGTTCCCGGGT
1743(compl)                                                             CGGTGAATACGTTCCCGGGC
```

**FIG. 89E**

```
E.colirrsE      1389 CTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCT
Cam.jejun5      1368 CTTGTACTCACCGCCCGTCACACCATGGGAGTTGATTTCACTCGAAGCCGGAATACT--A-A
Stp.aureus      1399 ATTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCT
1743(compl)          CTTGTAC

E.colirrsE      1451 TCG-GGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCG
Cam.jejun5      1427 AC---T-AGTTACCGTCCACAGTGGAATCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCG
Stp.aureus      1461 TTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGTGAAGTCGTAACAAGGTAGCCG

E.colirrsE      1512 TAGGGGAACCTGCGGTTGGATCACCTCCTTA---
Cam.jejun5      1485 TAGGAGAACCTGCGGTTGGATCACCTCCT-----
Stp.aureus      1523 TATCGGAAGGTGCGGCTGGATCACCTCCTTTCT-
```

**FIG. 89F**

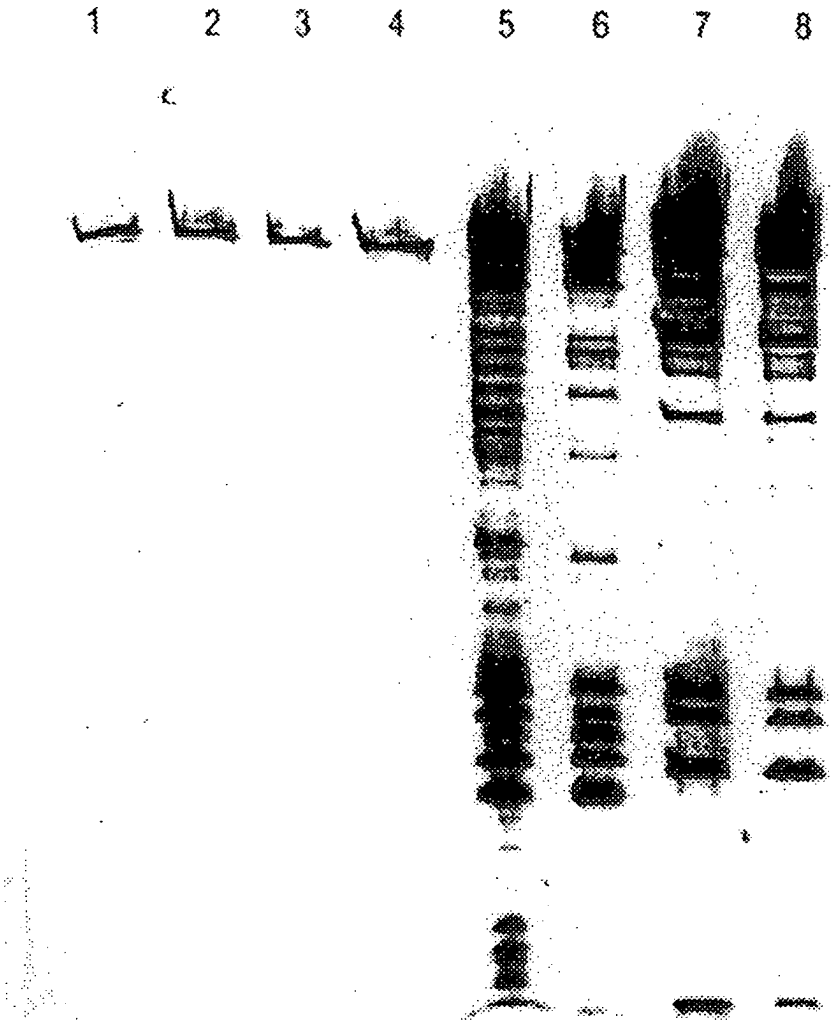



FIG. 90

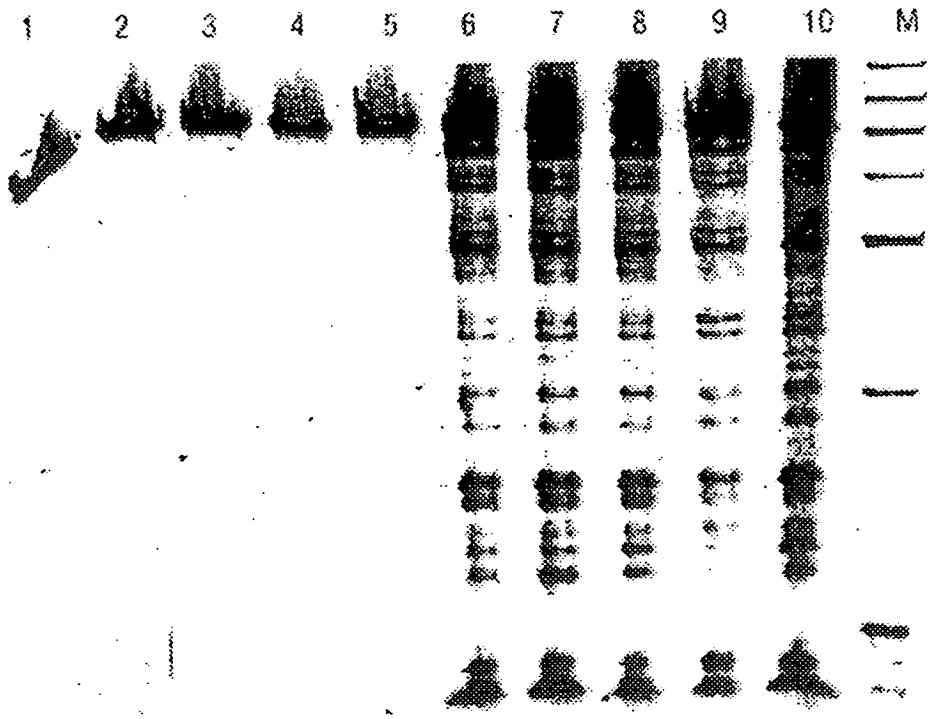



FIG. 91A

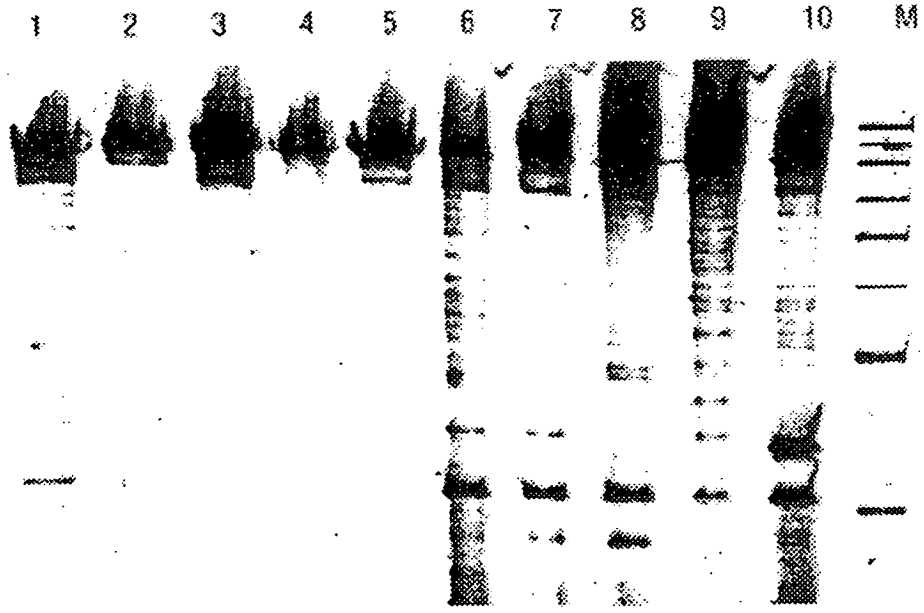



FIG. 91B

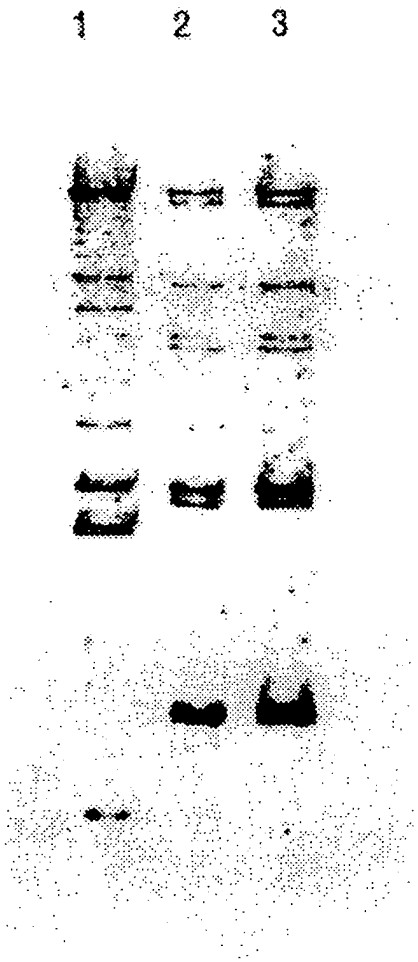



FIG. 92

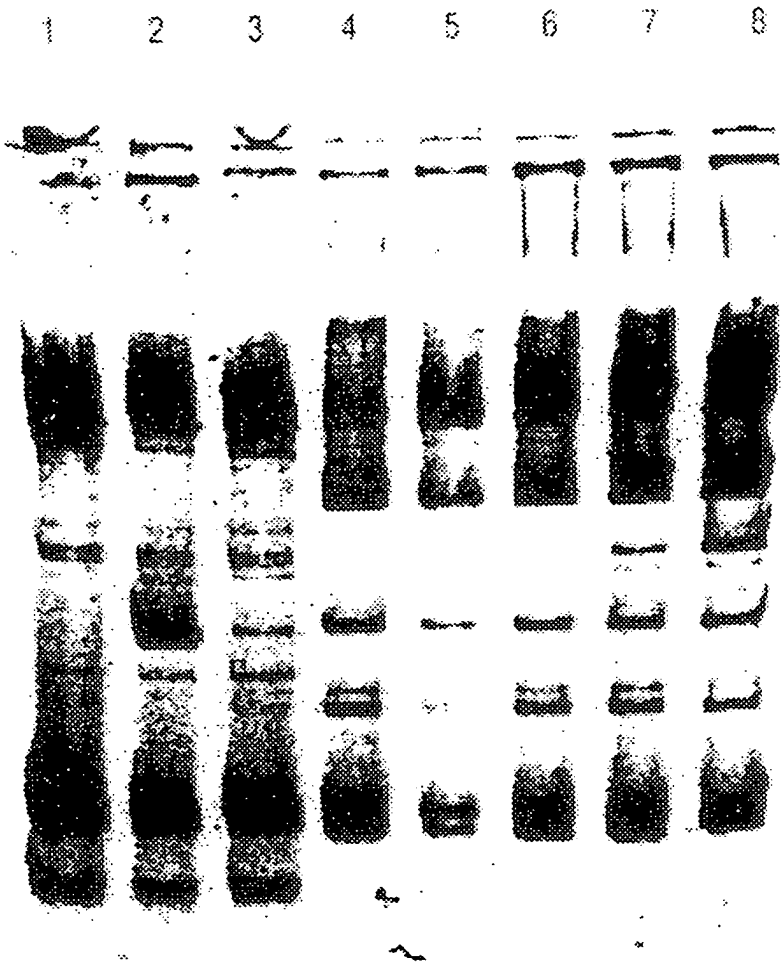



FIG. 93

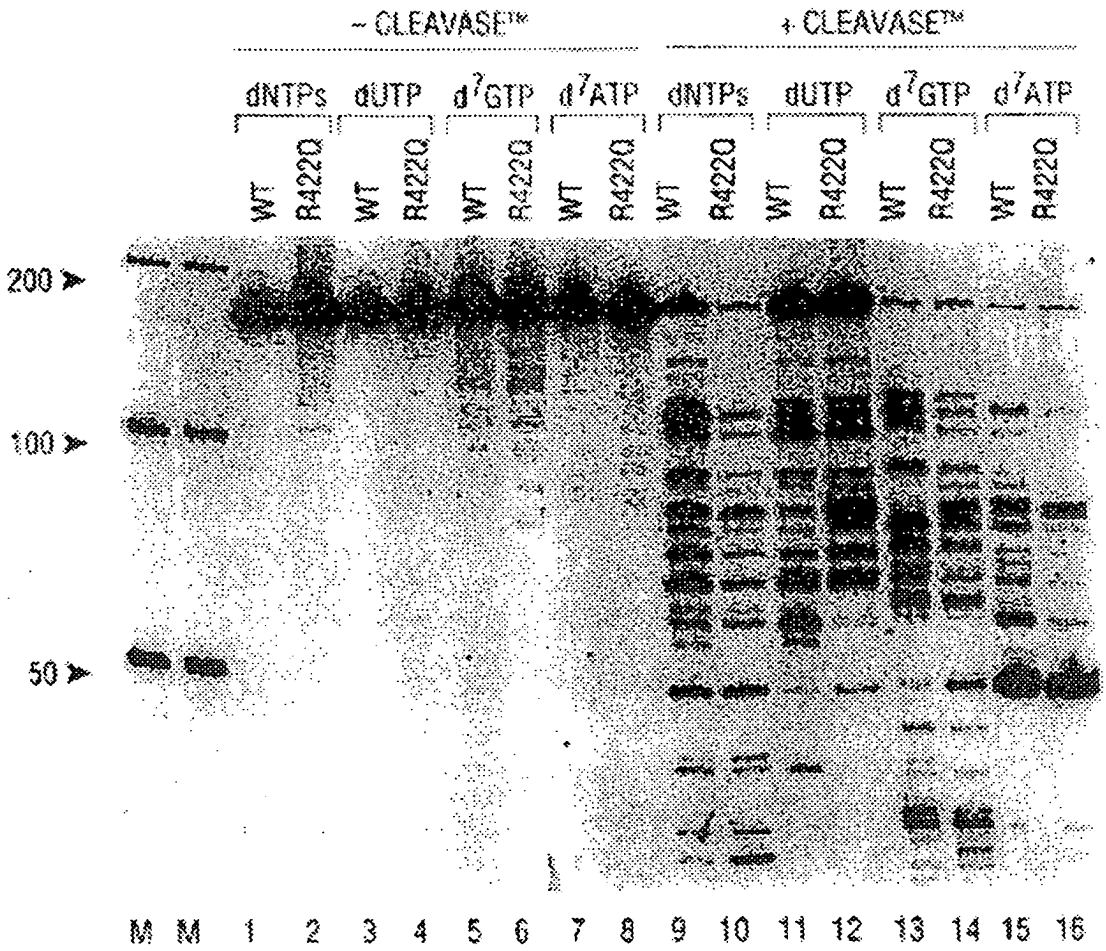

- CLEAVASE™
+ CLEAVASE™
dNTPs
dUTP
d7GTP
d7ATP
WT
R422Q
200
100
50
M M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

FIG. 94